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HYPHAL FUSIONS IN DERMATOPHYTES[1]

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Abstract

Hyphal fusions have been recognized as an important character of dermatophytes.

In *Microsporon audouini*, *M. lanosum*, and *Trichophyton gypseum* hyphal fusions: (1) are formed between hyphae of one and the same mycelium isolated from a single patient, (2) are formed between any two mycelia of the same species isolated from two different patients, and (3) are *not* formed between a mycelium of one species and a mycelium of another species.

The occurrence or non-occurrence of hyphal fusions between hyphae of two mycelia of different origin may be applied as a criterion for identifying species of dermatophytes whose specific nature is uncertain.

I. Introduction

As dermatologists are well aware, the taxonomy of the fungi which cause diseases of the skin is in a confused state. This is due in part to the fact that dermatophytes in general are Fungi Imperfecti which yield no perfect fruit-bodies, and in part to the inconstancy in the characters of the mycelium when grown for a considerable period of time. Hitherto, species have been distinguished from one another (1) by macroscopic appearance when grown on Sabouraud's medium and (2) by the spores and swellings which may be observed upon their individual hyphae (5, 8).

In 1931, Buller (3), in the fourth volume of his *Researches on Fungi*, called attention to the prevalence and physiological importance of the hyphal fusions in the mycelia of Ascomycetes, Basidiomycetes, and Fungi Imperfecti. He pointed out that, in many species of fungi, hyphal fusions may take place between two hyphae of one and the same mycelium derived from a single spore, or between a hypha derived from one monosporous mycelium and a hypha derived from another monosporous mycelium. Furthermore, in discussing the various functions of hyphal fusions, he showed that the formation of hyphal fusions need not have, and indeed very frequently does not have, any connection with sexual phenomena.

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In 1924, Buller (2) paired the diploid mycelium of *Panus stypticus** physiological form *luminescens* of North America with *P. stypticus* p.f. *non-luminescens* of England and Europe, and observed that hyphal fusions are formed between the two mycelia. He regarded the occurrence of these hyphal fusions as supporting the view that the two physiological forms of *P. stypticus*, which occur on opposite sides of the Atlantic Ocean, belong to one and the same species. Subsequent work, as yet unpublished, carried out by him with the assistance of Miss Ruth Macrae, has confirmed his conclusion; for it has been found that, when a haploid mycelium derived from a single spore of *P. stypticus* p.f. *luminescens* is paired with a haploid mycelium derived from a single spore of *P. stypticus* p.f. *non-luminescens*, a diploid clamp-connection-bearing mycelium results.

Buller's observations on hyphal fusions in fungi generally have suggested the following questions: are hyphal fusions present in dermatophytes; and, if so, of what value are they for diagnostic purposes? An attempt to answer these questions has been undertaken by the authors and the results of their investigations upon *Microsporon audouini*, *M. lanosum*, and *Trichophyton gypseum* are recorded in this paper.†

II. Microsporon audouini

(a) Methods

Natural Occurrence.—Of the 43 patients suffering from tinea capitis, who have come to the notice of the authors during the last six months, in more than half of them (65%) the condition was due to an infection by *Microsporon audouini* Gruby (Plate I, Fig. 1). Evidently, this fungus is the commonest cause of ringworm of the scalp in Winnipeg. This conclusion accords well with that of Adamson (1) who, in 1895, recorded that the frequency of ringworm cases in England due to *Microsporon audouini* was 80-90%.

Selection of Hairs.—Infected hairs from the head of a patient suffering from tinea capitis were selected by means of a water-cooled ultra-violet light apparatus equipped with a Wood's filter. This method was first introduced by Vigne (7). In the light obtained from the water-cooled lamp with the filter, the infected hairs show a green fluorescence and thus they can readily be distinguished from the healthy hairs.

Culture Media.—The infected hairs were planted in hanging drops of Sabouraud's medium or, less frequently, of sterile water. As was to be expected, the growth of the fungus was not so rapid in water as in Sabouraud's medium.

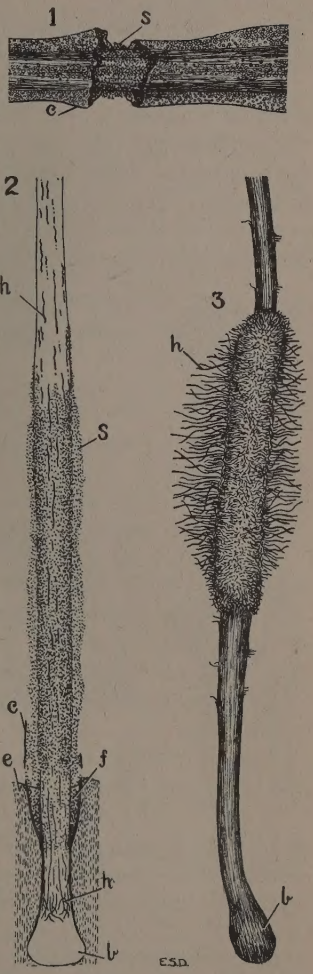
Two different modifications of Sabouraud's medium were employed. They did not contain the special maltose or peptone recommended by Sabouraud, but both gave satisfactory results. (1) Forty grams of pure commercial

**Panus stypticus is a wood-destroying gill-fungus. Its small bracket-like fruit-bodies occur on stumps of the birch. In the North American form both the mycelium and the fruit-bodies give out light. The English and European form is non-luminous in all stages of its development.*

†The authors gratefully acknowledge their indebtedness to Dr. Howard Fox and Dr. E. Muskatblit of the Department of Dermatology of New York University for carefully verifying their determination of the three species of fungi here named.

maltose, 10 gm. of standardized bacto-peptone, and 18 gm. of shredded agar-agar were dissolved in one litre of distilled water in an autoclave. The mixture was then filtered through paper and poured into containers. It was finally sterilized by allowing the pressure in the autoclave to rise slowly to 10 lb. upon three successive days. (2) An American commercial preparation of Sabouraud's medium manufactured by "Difco" was used. This contained 10 parts of bacto-peptone, 40 parts of bacto-dextrose, and 15 parts of bacto-agar. To make up this preparation, 65 gm. of the mixture was boiled with a litre of water, dispensed into containers, and sterilized in the same manner as before.

Culture Method.—Hairs infected with *M. audouini* become so packed with fungal spores beneath the cuticle (Text-fig. 1) that the cuticle is soon ruptured and peels off, thus exposing the sheath of spores (Text-fig. 2). The spore-sheath extends from below the level of the scalp to a distance of several millimetres above the follicle. When an infected hair is planted in a hanging drop of Sabouraud's medium or of water, the spores making up the sheath commence to germinate after about eight hours. Infected hairs were sown in flasks of Sabouraud's medium, and after 24 hr. the base of each hair could be seen with the naked eye to be surrounded by a white halo made up of hyphae radiating out from the sheath (Text-fig. 3 and Plate I, Fig. 2). Mycelia obtained from infected hairs were stored in test tubes and Erlenmeyer flasks containing Sabouraud's medium.



TEXT-FIGS. 1–3. Hairs from scalp infected with *Microsporon audouini;* magnification, 43. FIG. 1. Portion of a hair with its cuticle ruptured but still retained: *c*, cuticle of hair; *s*, fungal spores. FIG. 2. A hair in its follicle, after the loss of the cuticle: *b*, bulb of the hair; *c*, cuticle of the hair; *e*, epithelial tissue of the scalp; *f*, follicular cavity; *h*, fungal hyphae; and *s*, fungal spores. FIG. 3. A hair which had been in Sabouraud's medium a few days. The fungal spores have produced hyphae which have grown out into the nutrient medium: *b*, bulb of the hair; *h*, fungal hyphae.

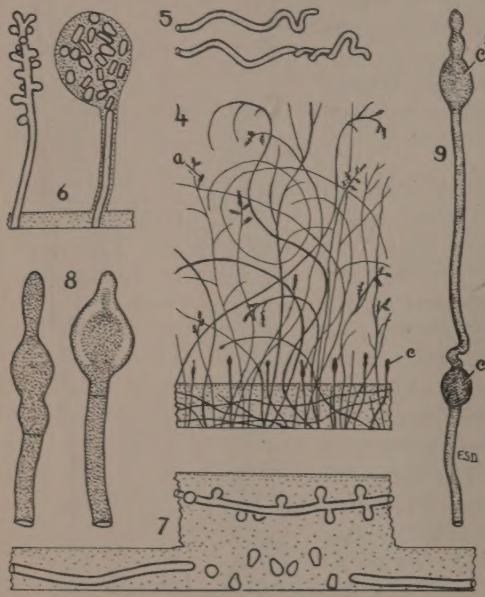
(b) THE MYCELIUM

The Young Mycelium.—The hyphae of *M. audouini* grow out from an infected hair or from a stock-culture inoculum into the medium to form a mycelium which remains in a vegetative condition for the first three or four days.

The hyphae grow at the rate of 0.25 to 0.5 mm. per day. The width of the hyphae varies from 3 to 6 μ. The smaller hyphae are often sinuous or wavy in outline and sometimes are spirally twisted into corkscrew shapes (Text-

fig. 5). When the mycelium is about two days old, one or two hyphal fusions may be observed. A detailed description of hyphal fusions will be given later.

After the mycelium has been growing in a hanging drop of Sabouraud's medium for three or four days, some of the hyphae commence to grow out of the medium into the air. The aerial mycelium developed in flask cultures forms a nap or down above the surface of the culture medium. As the aerial hyphae are not very long, the nap is close and extends only about a quarter of a millimetre above the level of the medium (Text-fig. 4 and Plate I, Fig. 3). It is these aerial hyphae which produce the spores.



TEXT-FIGS. 4–9. Aerial hyphae and spores of *Microsporon audouini;* the agar medium in Figs. 4, 6, and 7 has been stippled. Magnification: Fig. 4, 45; Figs. 5–9, 400. FIG. 4. Diagram of a lateral view of an aerial mycelium as it appears when a transverse section is made through the agar on which the fungus is growing: *a*, aleuriospores; *c*, chlamydospore. FIG. 5. Aerial hyphae, showing spiral twisting. FIG. 6. Aleuriospores borne upon aerial hyphae. The spores to the right have become detached and are floating in the drop of water collected on the conidiophore. FIG. 7. Hyphae with aleuriospores submerged in the medium. Part of the lower hypha has disintegrated and freed the spores. FIG. 8. Chlamydospores. FIG. 9. A hypha which has continued its growth through and beyond the chlamydospore c^1 and has produced a second chlamydospore c^2.

Aleuriospores.—When the mycelium has been growing in a hanging drop of Sabouraud's medium for about five days, some of the aerial branches (and to a less extent some of the submerged ones) form lateral conidia or aleuriospores (Text-fig. 4, *a*). These aerial conidia break off from the conidiophores and the conidiophores themselves segment. All the free cells which are thus formed frequently collect in liquid drops which appear on the aerial hyphae (Text-fig. 6). When the aleuriospores are borne on submerged hyphae, they are sometimes set free by the disintegration of the main hypha (Text-fig. 7).

Chlamydospores.—When the mycelium is about two weeks old, a large number of hyphae grow out into the air a short distance from the surface of the culture medium. These aerial hyphae are less than a quarter of the length of the aerial hyphae which form the aleuriospores and, at their apices, they form lemon-shaped swellings termed chlamydospores (Text-fig. 4, *c* and Plate I, Fig. 4). In spite of the implication of their name, the chlamydospores are not thick-walled and they have

never been observed to become detached. For the present, therefore, they cannot be regarded as organs of dissemination. Growth is sometimes renewed at the base of the chlamydospores: a hypha grows through the old swelling, emerges at the swelling's apex, and then continues its growth in the culture medium (Text-fig. 9).

Hyphal Fusions.—Hyphal fusions were observed: (1) between two hyphae of one and the same mycelium derived from a single hair; and (2) between two hyphae which originated from two mycelia derived from hairs of different patients.

(1) A hair of patient A was placed in Sabouraud's medium in a flask and a mycelium of *M. audouini* was obtained from it. This mycelium was grown in a conical flask for three months. At the end of this time, a small pin-head mass of the aerial mycelium was removed from the flask and was set in the middle of a hanging drop of Sabouraud's medium in a van-Tieghem cell the bottom of which was covered with a shallow layer of sterile water. In the course of about two days the hyphae grew out from the inoculum into the culture medium. The hanging drop was about 3 mm. in diameter. The mycelium grew out radially and, in the course of three days, attained a diameter equal to that of the drop. It then pushed out beyond the drop into the film of water which had been formed by condensation on the cover-glass.

Five days after inoculation, hyphal fusions were observed both within the culture medium and in the film of water surrounding it. The fusions within the medium were relatively few and were observed only after careful search. On the other hand, the fusions between hyphae in the film of moisture were very numerous and could be determined with ease. In one particular part of the mycelium growing in the water-film, having a length of 1 mm. and a breadth of 0.2 mm., nine hyphal fusions had been established. Hyphal fusions, seen in the area of the mycelium to which reference has just been made, are shown in Text-figs. 10, 11 and 12.

From an examination of Text-figs. 10, 11 and 12 it appears that all the fusions there illustrated were formed between the end of one hypha and the lateral wall of another hypha. This type of hyphal fusion has been described and illustrated by Buller (3).

From an examination of the photograph of the mycelium of *M. audouini* in Plate I, Fig. 5, it can be seen that the hyphal fusion there illustrated probably was formed as follows. Two hyphae happened to be parallel and then, at a point where the two hyphae were close together, they sent out short peg-like protuberances which grew directly towards each other and fused apically. In Plate I, Fig. 5, near the middle of the uppermost pair of hyphae, two of these peg-like protuberances can be seen before they have come into contact.

In a mycelium growing out into the water-film around a hanging drop of culture medium, more and more hyphal fusions were formed as the mycelium extended in area. Finally, at the end of about two weeks after inoculation, by which time the culture medium had become exhausted, the number of hyphal fusions was often 100 or more. It seems probable that the formation of hyphal fusions is favored by starvation.

Similar observations to those just recorded were made with each of two mycelia of *M. audouini* isolated from single hairs of two other patients, B and C, also suffering from tinea capitis.

(2) Mycelia of *M. audouini* derived from the patients A, B and C were now grown together in pairs. The pairs were A-B, A-C, and B-C. The three pairs were established in as many hanging drops of Sabouraud's medium which were similar to those described above. To make a pair, a pin-head mass of the aerial hyphae of one of the mycelia was set near the middle of the drop and then a similar mass from another mycelium was set in the drop 1-2 mm. from the first mass (Text-fig. 13, *a*).

The two inocula in each hanging drop grew out into the medium in the usual way (Text-fig. 13, *b*) and then out into the film of water on the cover-glass. The hyphae of the two mycelia along the line of contact soon came to cross one another at various angles (Fig. 13, *c*).

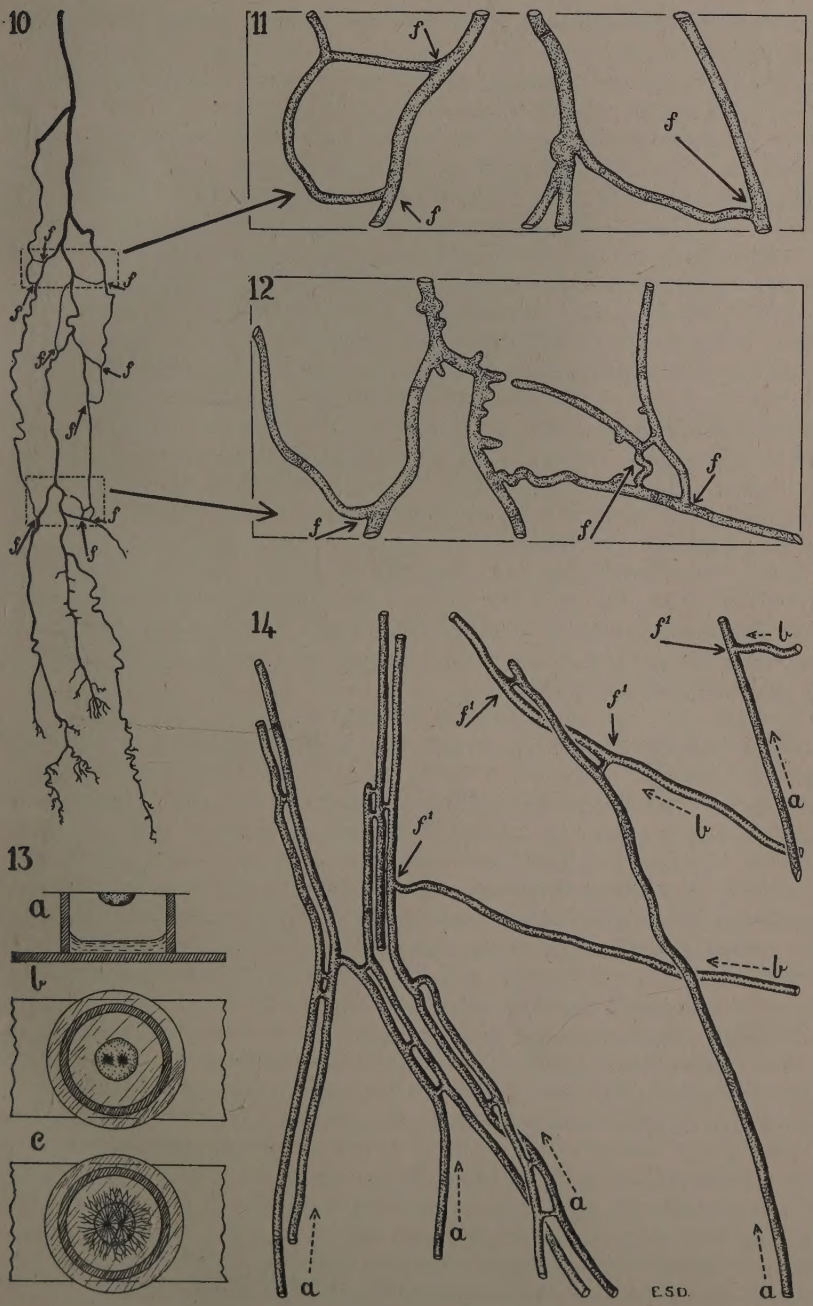
In each of the three hanging drops, hyphal fusions were sought for between a hypha derived from one mycelium and a hypha derived from the other mycelium, and they were readily found in the film of water outside the medium (Text-fig. 14 and Plate I, Fig. 6). Scores of such hyphal fusions between the two mycelia in each of the three pairs were observed. Convincing evidence was thus obtained that *hyphal fusions readily take place between two mycelia of Microsporon audouini, one derived from a hair of one patient and the other derived from a hair of another patient.*

III. Microsporon lanosum

(a) Methods

Natural Occurrence.—Microsporon lanosum Sab. is a parasite of the hair and the skin. On the scalp it results in crusted lesions, and on the glabrous skin it forms circular, red, scaly lesions (Plate II, Fig. 1). At Winnipeg, after *M. audouini*, *M. lanosum* is the commonest cause of ringworm of the scalp. Seven of the 56 children with ringworm who were under observation during the last six months were infected with *M. lanosum*.

Text-figs. 10–14. Hyphal fusions in the mycelium of *Microsporon audouini*. Fig. 10. Some of the peripheral hyphae of a mycelium which had been growing in a hanging drop of Sabouraud's medium for a month. The hyphae have grown out of the medium and are in the water condensed on the cover-slip: *f f f*, fusions between hyphae of the same mycelium. Magnification, 70. Figs. 11 and 12. Portions of Fig. 10, drawn more highly magnified: *f f f*, fusions between hyphae of the same mycelium. Magnification, 400. Fig. 13. Diagrams to show the materials and methods used to determine whether or not hyphal fusions are formed between different mycelia: *a*, inocula of the two mycelia to be tested have been planted side by side in a hanging drop of nutrient medium in a van-Tieghem cell; *b*, the inocula have commenced to grow; *c*, the two mycelia from the inocula have grown out of the medium into the water condensed on the cover-glass where they have met and crossed each other. It is in the parts of the culture where the two mycelia have crossed each other in the water-film outside the culture medium that fusions can most readily be found. Natural size. Fig. 14. Hyphal fusions between two mycelia of *M. audouini*. Two mycelia, *a a a* and *b b b*, obtained from patients A and B respectively, have been grown together in a hanging drop of culture medium (*cf.* Fig. 13) for two weeks. The arrows with broken shafts by the letters *a a a* and *b b b* indicate the direction of growth of the hyphae. The hyphae of the mycelium *a a a* have met with, and have fused with, the hyphae of the mycelium *b b b* at four places each of which is indicated by an arrow and the letter f^1. Magnification, 400.



TEXT-FIGS. 10-14.

Sartory (6) considers that *M. lanosum* is the most common dermatophyte of animal origin.

Selection of Infected Tissue.—The mycelium was obtained either from infected hairs or from infected skin. The infected hairs were selected by means of the water-cooled ultra-violet light with Wood's filter attachment. Infected hairs examined in this light gave out a green fluorescence. The infected skin which was used consisted of epidermal scales selected from the margins of the skin lesions. This tissue was always penetrated by fungal hyphae (Text-fig. 15).

Culture Media.—The culture media employed for growing *M. lanosum* were the two modifications of Sabouraud's medium already described.

Culture Method.—Hairs or epidermal scales infected with *M. lanosum* were placed in hanging drops of Sabouraud's medium. A day or two after an infected hair had been placed in a hanging drop, a mycelium could be seen growing out from the region of the hair that had previously been observed to be covered by a sheath of fungal spores. A day or two after an infected epidermal scale had been planted in a hanging drop, a mycelium could be seen growing out from every part of the surface of the scale. The mycelium was allowed to continue its growth for a week or two, after which it was transferred to flasks or test tubes of Sabouraud's medium.

Similar morphologically and characteristic of *M. lanosum* were: (1) five mycelia obtained from as many different patients suffering from tinea capitis and tinea corporis; and (2) two mycelia obtained from the same patient, one mycelium taken from a lesion on the scalp and the other taken from a lesion on the glabrous skin of the body.

(b) The Mycelium

The Young Mycelium.—The mycelium of *M. lanosum* grew from the infected hairs or epidermal scales into hanging drops of Sabouraud's medium at the rate of 0.3-0.5 mm. per day. Within about four days after tissue infected with *M. lanosum* has been placed in the culture medium, large numbers of aerial hyphae grow out from the medium down into the air in the van-Tieghem cell and there attain a length of several millimetres. The aerial hyphae are inclined to twine around each other so as to form twisted strands (Text-fig. 16). The profuse growth of aerial mycelium characteristic of *M. lanosum* may be seen not only in van-Tieghem cells but also in test tubes and flasks, and it gives the culture a deep, white, felted covering or "duvet" (Plate II, Fig. 3).

Spindles.—After about a week of growth either in hanging drops or in test tubes or flasks, short aerial hyphae produce spindle-shaped multilocular spores called "spindles", "fuseaux", or "macroconidia" (Text-figs. 16 *s*, 19 and Plate II, Figs. 2, 4). The spindles can be obtained by tearing away the web of long aerial hyphae from a culture with a needle and then scraping the surface of the agar. The spindles adhere to the needle. By no means are they always present in *M. lanosum* cultures, for they were observed in only five of twenty-five or more hanging-drop cultures and in only two of five or more cultures growing in flasks. It was not until the two cultures in flasks had been kept for about four months that they began to form spindles, and two months later no spindles could be found in either of them.

Spindles (Text-fig. 19 and Plate II, Figs. 2, 4) are developed aerially. They may arise singly or be borne in clusters upon a branched hypha. Usually they are slender and attenuated at the apex, and sometimes they end in a terminal bristle. The spore-coat bears small wart-like protuberances on its outer surface. The cavity is divided transversely into about nine segments (Fig. 19).

As a mycelium develops, the form of its spindles may change. One particular mycelium, when four months old, possessed small blunt-ended spindles made up of six or even fewer segments, while, two months later, it had begun to form large pointed spindles made up of about nine or more segments. A second mycelium possessed normal spindles when it was three weeks old but, after three more months, the spindles were small and degenerate and possessed no septa. This variation in the form of spores has been noticed by other authors (4, 8), and it is noteworthy because the classification of some dermatophytes is largely based on the character of the spores.

Chlamydospores.—When the mycelium has been growing about three weeks in a flask or test tube, terminal chlamydospores are sometimes formed. These chlamydospores are similar to those of *M. audouini* but are not so abundant.

Conidia.—Some of the longer aerial hyphae may become divided into short segments by transverse walls (Text-figs. 16 *c*, 17, and 18). These segments become detached from the hyphae and act as conidia. They swell in water and become irregular in shape.

Intercalary Swellings.—The older submerged hyphae of *M. lanosum* become swollen into oval nodes at intervals along their length (Text-fig. 21, *i.s.*).

Hyphal Fusions.—Hyphal fusions were observed: (1) between two hyphae of one and the same mycelium derived from a single hair; and (2) between two hyphae which originated from two mycelia derived from hairs of different patients.

(1) A hair from patient D was placed in Sabouraud's medium in a flask and a mycelium of *M. lanosum* was obtained from it. This mycelium was grown in the flask for upwards of three months. At the end of this time, a small pin-head mass of the aerial mycelium was removed from the flask and set in the middle of a hanging drop of Sabouraud's medium in a van-Tieghem cell as used in a similar experiment with *M. audouini*. In the course of about two days, the hyphae grew out from the inoculum into the culture medium. The mycelium grew radially and, in the course of three days, attained a diameter equal to that of the drop. It then pushed out beyond the drop into the film of water which had been formed by condensation on the cover-glass.

Five days after inoculation, large numbers of hyphal fusions were observed both within the culture medium and in the film of water surrounding it (Text-figs. 20, 21 and Plate II, Fig. 5).

Observations similar to those just recorded were made with each of two mycelia of *M. lanosum* isolated from single hairs from two other patients, patients E and F, also suffering from tinea capitis and tinea corporis.

(2) Mycelia of *M. lanosum* derived from the patients D, E and F were now grown together in pairs. The pairs, which were D-E, D-F, and E-F, were established in hanging drops of Sabouraud's medium. To make a pair, a

pin-head mass of the aerial hyphae of one of the mycelia was set near the middle of the drop and then a similar mass from another mycelium was set in the drop 1-2 mm. from the first mass (Text-fig. 13, *a*).

The two inocula in each hanging drop began to grow into the medium after the first or second day (Text-fig. 13, *b*) and then into the film of water on the cover-glass. About ten days after inoculation, the hyphae of the two mycelia had come into contact and had commenced to cross each other at various angles (Text-fig. 13, *c*).

In each of the three hanging drops, hyphal fusions were sought for between a hypha derived from one mycelium and a hypha derived from the other mycelium, and they were readily found in the film of water outside the medium (Plate II, Fig. 6). Large numbers of such hyphal fusions between the two mycelia in each of the three pairs were observed. These observations, together with identical results obtained from a repetition of the experiments, afford convincing evidence that *hyphal fusions readily take place between two mycelia of Microsporon lanosum, one derived from the tissue of one patient and the other derived from the tissue of another patient.*

IV. Trichophyton gypseum

(a) Methods

Natural Occurrence.—During the last six months, the authors examined twelve adult male patients with fungal diseases, and three of the twelve patients were found to be suffering from sycosis parasitaria (tinea barbae). *Trichophyton gypseum* Bodin (= *T. asteroides* Sab.) was isolated from the beard-hairs of one of the three patients, patient G, who is shown in Plate III, Fig. 1.

In patient G, the hairs of the beard area, particularly on the upper lip, were infected. An inflammatory reaction had been set up and pus was exuding from the hair follicles. Microscopical examination showed that the shaft and follicular sheath of each diseased hair had been penetrated by fungal hyphae (Plate III, Fig. 2, A).

Selection of Hairs.—The faces of patients with sycosis parasitaria were examined with the ultra-violet light apparatus, but none of the diseased beard-hairs gave out a green fluorescence. Ultra-violet light was therefore of no assistance in selecting hairs infected with *T. gypseum*. To obtain a hair which would give rise to a mycelium, it was necessary to remove large numbers of hairs from the diseased areas and place them upon Sabouraud's medium.

Culture Medium and Culture Method.—The culture media employed for

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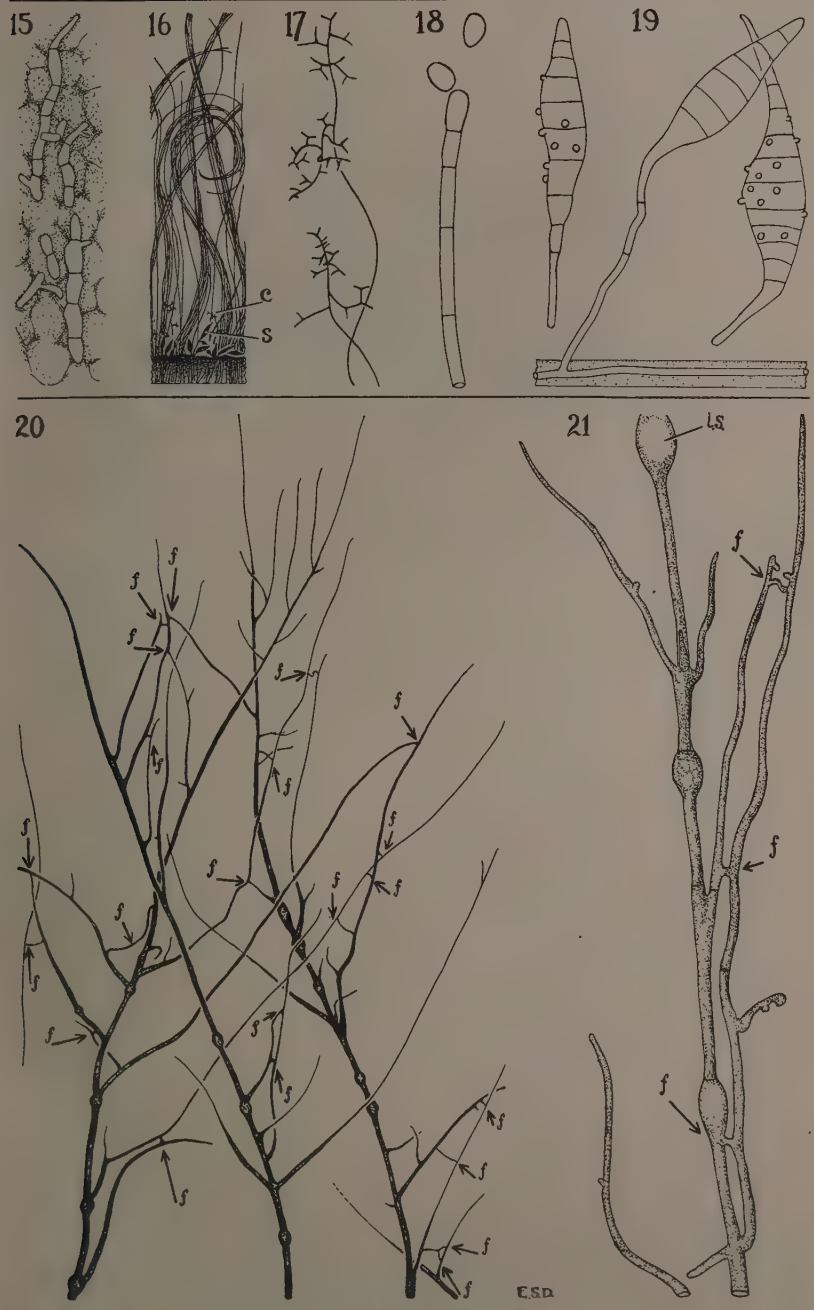
Text-figs. 15–21. The mycelium and spores of *Microsporon lanosum*. Fig. 15. A piece of an epidermal scale from patient D, penetrated by fungal hyphae. Magnification, 400. Fig. 16. A diagram of part of an aerial mycelium produced upon Sabouraud's medium, seen in lateral view: at the base, the culture medium; *c*, conidiophore; *s*, spindle. Magnification, 42. Fig. 17. Conidiophores and conidia. Magnification, 70. Fig. 18. Conidiophore and conidia, more highly magnified. Magnification, 400. Fig. 19. Spindles. Magnification, 400. Fig. 20. Mycelium from patient F, grown in a hanging drop of Sabouraud's medium for ten days: *f f f*, numerous hyphal fusions. Magnification 70. Fig. 21. Mycelium from patient E, grown in a hanging drop of Sabouraud's medium for ten days: *f f f*, hyphal fusions; *i.s.*, one of three intercalary swellings on a main hypha. Magnification, 400.



TEXT-FIGS. 15-21.

growing *T. gypseum* were the two modifications of Sabouraud's medium already described.

Beard-hairs infected with *T. gypseum* were placed in hanging drops of Sabouraud's medium. After a day or two some of the hairs had given rise to a mycelium. All of the mycelia were contaminated with bacteria from the pus adhering to the hair, so that it was necessary to separate the fungus from the bacteria. This was accomplished by transferring with a sterile needle a pin-head mass of aerial mycelium from the hanging drop to a flask of culture medium.

(b) The Mycelium

Spindles.—The mycelium of *T. gypseum* from patient G was allowed to grow in the flask for three weeks. It attained a diameter of a few centimetres and produced a dense, dead-white, aerial mass of hyphae. When a portion of the aerial mycelium was pulled away with a needle and examined microscopically it was found to contain a number of spindles of characteristic appearance. They were club-shaped with rounded ends, were about 20 μ long and 5 μ wide, and possessed two or three cross-walls (Plate III, Fig. 2, B).

The aerial mycelium, when three weeks old, in addition to spindles contained many hyphae that were twisted into close spirals.

Aleuriospores.—When the mycelium of *T. gypseum* had been in culture nearly a month, the spindles were no longer to be seen and the aerial hyphae were observed to be producing large numbers of aleuriospores (Plate III, Fig. 4). The aleuriospores of this species, unlike those of *Microsporon audouini*, tend to be constricted at their base. They are arranged laterally on the hyphae, and, when they are crowded together, the inflorescence has the appearance of a bunch of grapes.

By the time the culture was about a month old, the contour of the mycelium had become definitely star-shaped.

Chlamydospores.—As the medium upon which the fungus was growing became progressively exhausted, the texture of the surface of the mycelium became granular and plaster-like (Plate III, Fig. 3). Microscopical examination showed that this granular appearance was due to the aerial hyphae having swelled up at intervals along their length so as to form chains of intercalary chlamydospores. The chlamydospores were spherical and the largest were 14 μ in diameter. The aerial hyphae of a four-months-old culture was made up almost entirely of chlamydospores.

When transfers of the fungus were made from an exhausted medium to a fresh medium, spindles and aleuriospores were again obtained.

Pleomorphism.—When a culture of *T. gypseum* was about six months old, a white downy growth of sterile aerial hyphae commenced to grow over its surface. This change is known in the literature as "pleomorphism." Pleomorphism is characteristic of dermatophytes and, according to our present knowledge, it is an irreversible change.

Hyphal Fusions.—Hyphal fusions were observed: (1) between two hyphae of the same mycelium derived from a single hair; and (2) between two hyphae which originated from two mycelia derived from hairs of different patients.

(1) A hair from patient G was placed in Sabouraud's medium in a flask and a mycelium of *T. gypseum* was obtained from it. When the mycelium had been growing in the flask for four months, a small pin-head mass of the aerial mycelium was removed from the flask and set in the middle of a hanging drop of Sabouraud's medium in a van-Tieghem cell, the bottom of which was covered with a shallow layer of sterile water. In the course of two days, the hyphae grew out from the inoculum into the culture medium. It then attained a diameter equal to that of the drop, and then pushed out beyond the drop into the film of water which had been formed by condensation on the cover-glass.

About a week after inoculation, a few hyphal fusions were found within the drop of culture medium, and many in the film of water surrounding it (Plate III, Fig. 5).

Observations similar to those just recorded were made with a mycelium of *T. gypseum* obtained from another patient, H, who was also suffering from sycosis parasitaria. The material from patient H was obtained in New York by Dr. Muskatblit.

(2) The mycelium of *T. gypseum* derived from patient G in Winnipeg was then grown with the mycelium of *T. gypseum* sent from New York and derived from patient H. The pair was established in a hanging drop of Sabouraud's medium. To make the pair, a pin-head mass of the aerial hyphae of mycelium G was set near the middle of the drop and then a similar mass of mycelium H was set in the drop 1-2 mm. from the first mass (Fig. 13, *a*).

After the two mycelia had crossed each other, hyphal fusions were sought for between a hypha derived from one mycelium and a hypha derived from the other. After 13 days, seven or eight hyphal fusions between mycelia G and H were discovered along the margin of the medium (Plate III, Fig. 6). This experiment goes to show that *hyphal fusions readily take place between two mycelia of Trichophyton gypseum, one derived from a hair of one patient and the other from a hair of another patient*, even though these patients are from widely separated geographical areas.

V. Pairings between Mycelia of Different Species

(a) Microsporon audouini with M. lanosum

Six mycelia were employed. Three of them were *M. audouini* obtained from patients A, B and C, and the other three were *M. lanosum* obtained from patients D, E and F.

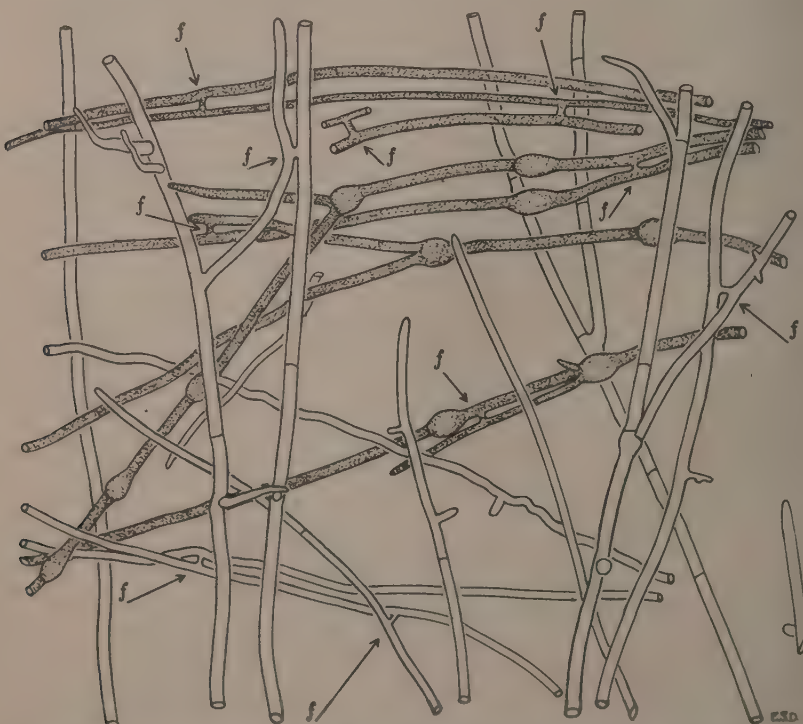
The mycelia were grown in hanging drops in pairs in the manner already described. Pairings between the mycelia of *M. audouini* and the mycelia of *M. lanosum* were made in all possible ways. Two hanging-drop cultures were made of each pair. Table I embodies the results obtained.

The appearance of the paired mycelia B-E in experiment No. 5 after one month is shown in Text-fig. 22. The hyphae of *M. lanosum* are characterized by intercalary swellings, while those of *M. audouini* can be distinguished by a slight tendency to waviness. In Text-fig. 22, to aid the eye in distinguishing the two mycelia, the mycelium of *M. lanosum* has been shaded while that of

TABLE I

Results of pairing mycelia of *Microsporon audouini* with mycelia of *M. lanosum*

Experiment No.	Pairs of mycelia	Date of inoculation	Result of examinations of the preparations made at intervals up to May 22
1	A – D	April 25	
2	A – E	April 25	
3	A – F	April 25	In each of the nine pairings fusions were observed between hyphae of the same mycelium but never between hyphae belonging to the two different mycelia
4	B – D	April 16	
5	B – E	April 25	
6	B – F	April 25	
7	C – D	April 25	
8	C – E	May 5	
9	C – F	May 5	



Text-fig. 22. Absence of hyphal fusions between the mycelia of two species of Microsporon. The mycelium of *Microsporon audouini* from patient C, left *unshaded*, and the mycelium of *Microsporon lanosum* from patient E, *shaded*, which have been growing side by side in a hanging drop of Sabouraud's medium for three weeks, have come into contact with one another, have become interlaced, but have *not fused* with one another. Hyphal fusions may be observed between two hyphae of *M. audouini* or between two hyphae of *M. lanosum* but not between a hypha of *M. audouini* and a hypha of *M. lanosum*: *f f f*, numerous hyphal fusions, but none of them between a hypha of *M. audouini* and a hypha of *M. lanosum*. Magnification, 400.

M. audouini has been left unshaded. It can be seen that there is an abundance of fusions between hyphae of the same species, but that there are no fusions between hyphae of different species.

As indicated in Table I, in all of the nine experiments, while fusions could be observed between hyphae belonging to one and the same mycelium of either *M. audouini* or *M. lanosum*, *no fusions could be observed between a hypha of M. audouini and a hypha of M. lanosum*.

(b) Trichophyton gypseum with Other Species of Dermatophytes

(1) *Trichophyton gypseum with Microsporon audouini*.—Three mycelia were employed. Two of them were *M. audouini* obtained from patients A and B and the other was *T. gypseum* obtained from patient G.

Two experiments were made. One hanging drop was inoculated with *T. gypseum* from patient G and *M. audouini* from patient A; and another hanging drop was inoculated with *T. gypseum* from patient G and *M. audouini* from patient B.

Three weeks after the two experiments had been set up, the hanging drops were examined microscopically and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *M. audouini*, *no fusions could be observed between a hypha of T. gypseum and a hypha of M. audouini*.

(2) *Trichophyton gypseum with Microsporon lanosum*.—Four mycelia were employed. Three of them were *M. lanosum* obtained from patients D, E and F, and the fourth was *T. gypseum* obtained from patient G.

The mycelia were grown in hanging drops in pairs in the manner already described. Pairings between the mycelia of *T. gypseum* and *M. lanosum* were made in all possible ways. Four hanging-drop cultures of each pair were set up.

Three weeks after the experiments had been started, the preparations were examined microscopically and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *M. lanosum*, *no fusions could be observed between a hypha of T. gypseum and a hypha of M. lanosum*.

(3) *Trichophyton gypseum with T. granulosum*.—Two mycelia were employed. One of them was *T. gypseum* obtained from patient G, and the other was *T. granulosum* Sab. obtained in New York by Dr. Muskatblit from patient I.

The mycelia were grown together in three hanging drops in the manner already described.

Three weeks after the experiments had been set up, the preparations were examined microscopically and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *T. granulosum*, *no fusions could be observed between a hypha of T. gypseum and a hypha of T. granulosum*.

T. granulosum is almost identical botanically with *T. gypseum*, and the two species are placed together by systematists (6) in the "Gypseum Group". That

hyphal fusions are not formed between these species is therefore of particular interest.

(4) *Trichophyton gypseum and Epidermophyton interdigitale.*—Two mycelia were employed. One was *T. gypseum* obtained from patient G, and the other was *E. interdigitale* Priestley obtained in New York by Dr. Muskatblit from patient J.

The mycelia were grown together in three hanging drops in the manner already described.

Three weeks after the experiments had been set up, the preparations were examined microscopically, and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *E. interdigitale*, *no fusions could be observed between a hypha of T. gypseum and a hypha of E. interdigitale.*

Cultures of *T. gypseum* and *E. interdigitale* on artificial media cannot be distinguished by experts, either by the naked eye or by a microscopic examination of the mycelia and their spores. They can be distinguished only clinically, *i.e.* by their parasitic behavior. *T. gypseum* attacks the beard-hairs, while *E. interdigitale* is restricted to the glabrous skin.

In spite of the very close resemblance of the two fungi, the lack of hyphal fusions between them in the experiment just recorded may be taken as evidence that they are indeed two different species.

VI. The Value of Hyphal Fusions for Identifying Species of Dermatophytes

It has been shown for the dermatophytes so far examined that: (1) hyphae belonging to the same mycelium fuse readily with one another; (2) when any two mycelia of different origin but of the same species are grown side by side, hyphal fusions are formed between hyphae of one mycelium and hyphae of the other; and (3) no hyphal fusions are formed between mycelia of different species.

In the light of the facts which have just been given, it is possible to apply a new method in the identification of dermatophytes. This method may be described as follows.

A mycelium of the fungus which one desires to identify is isolated from the diseased tissue of a patient and is grown in a culture medium. In the laboratory there must be stock cultures of a series of species of dermatophytes which have been properly identified. Then small pin-head masses of the unidentified mycelium are paired with similar masses of the mycelia of identified species in a series of hanging drops of culture medium suspended in as many van-Tieghem cells. The identified species chosen for the pairings are those which have produced in patients conditions clinically similar to those produced by the unidentified fungus. Let A, B, C, D and F be the identified species and X the unknown species used in the experiments. Then we have the following pairings: A-X, B-X, C-X, D-X, and E-X. At the end of about three weeks the cultures are examined with the microscope for hyphal fusions. Then it

will be found that hyphal fusions between the two mycelia in a van-Tieghem cell are present in one of the pairings but not in any of the others. From this result one may conclude that the species under investigation is identical with the one with which it forms hyphal fusions. If, for example, hyphal fusions between the two mycelia were found to be present in the pairing C-X but not in the pairings A-X, B-X, D-X, or E-X, such a result would indicate that the species X is identical with the species C.

By the method just described certain species of dermatophytes—those which form hyphal fusions—can be identified within a few weeks without the investigator waiting, often for months, for the characteristic spores to appear.

The presence or absence of hyphal fusions in pairing experiments has enabled the authors to determine the fungi responsible for the skin condition of (1) patient F and (2) patient H.

(1) Patient F, aged ten years, suffering from tinea corporis, showed scaling lesions one to two inches in diameter on the chest, back, and forehead. Each lesion was marked at the periphery by a circle of white vesicles. Examination of the child's body with the ultra-violet light and Wood's filter showed that occasional hairs on the lesions gave out a green fluorescence. Circular scaling lesions, such as those exhibited by patient F, may be caused by any one of several different dermatophytes.

A hair that was fluorescent in ultra-violet light was removed and stained with the gentian-violet stain combination for demonstrating fungi. An examination with the microscope revealed a mycelium within the hair and a mass of spores outside the hair. When epidermal scales from the lesions were treated with gentian violet, branching hyphae could be seen penetrating the tissue.

Infected epidermal scales were planted on Sabouraud's medium, and a mycelium appeared upon the medium two days later. The mycelium increased in size and took on a yellow color but remained perfectly sterile.

At the end of several weeks, the patient had been cured by iodine applications and had been discharged from the hospital, but the fungus which caused the disease was still undetermined.

It was not until after the cultures had been kept in the laboratory for four months that any spores were produced. In the fourth month the aerial mycelium produced smooth blunt-ended spindles with four or five cross-walls. At this stage of development, to the naked eye the mycelium resembled that of *Microsporon lanosum*, but the spindles were too small, had too few compartments, and were too blunt-ended to be typical for that species.

Experiments were then set up to determine whether or not hyphal fusions would take place between the mycelium derived from patient F and a mycelium from a culture of *M. lanosum* that had been definitely identified. After a few days, an abundance of fusions could be observed between the two mycelia, so that the unknown fungus was taken to be *M. lanosum*.

The determination of the mycelium from patient F as being *Microsporon lanosum* was substantiated by changes in the stock culture during the next month. The yellow color of the mycelium was gradually lost. The old

spindles fell away and were replaced by new ones. The new spindles were larger than the old ones, their tips were attenuated, their outer walls were ornamented with nodules; internally they were divided by 8-9 septa and, in general, their appearance was that of the spindles of *Microsporon lanosum*.

Thus it was not till after a lapse of six months that the fungus from patient F was satisfactorily identified by its spores, while by hyphal-fusion experiments it could have been identified a week or two after it was isolated.

It may be added that the mycelium of patient F was paired with three different mycelia of *Microsporon audouini*, with two different mycelia of *M. lanosum*, and with one mycelium of *Trichophyton gypseum* and that hyphal fusions were formed only between the mycelium from patient F and the two mycelia of *M. lanosum*.

(2) Patient H, a farm laborer, aged 19, suffering from sycosis parasitaria, showed a deep suppurating encrusted lesion two or three inches in diameter on the neck in the beard area.

Hairs were removed from the lesion and soaked in 8% caustic potash. When the hair tissue began to dissolve, a mycelium was distinctly visible both inside and outside the cuticle. The hyphae were composed of short segments which could easily be separated and they gave the appearance of a string of beads, thus suggesting that the mycelium was that of a *Trichophyton*.

A number of hairs were removed from the lesion and planted in Sabouraud's medium, and a mycelium developed from one of the hairs. The mycelium increased in size, but produced no spores even after the culture had been growing for several weeks.

In the meantime, the lesion responded readily to treatment and the patient was discharged from the hospital, but the fungus which caused the disease was still undetermined.

Experiments were then set up to find out whether or not the mycelium derived from patient H would form hyphal fusions with a mycelium from a stock culture of *Trichophyton gypseum*. Although the paired mycelia were kept growing in van-Tieghem cells for several weeks, no fusions whatsoever could be found between the unknown mycelium and the mycelium of *Trichophyton gypseum*. The negative results of these hyphal-fusion experiments led the authors to believe—contrary to their expectation— that the unknown fungus was not *Trichophyton gypseum*.

Lack of type cultures of other ectothrix *Trichophyta* prevented the authors from making further hyphal-fusion experiments, but a month later the mycelium took on a rose color and accorded very well with Sartory's description of *T. radiolatum*, Sab. (6).

According to Grigoraki (4) and others, all dermatophytes go through progressive stages of degeneration to sterility in which state it is impossible to distinguish species from one another. It remains to be shown whether or not these fungi in their sterile condition can be determined by hyphal-fusion experiments.

Finally, it is suggested that the hyphal-fusion method described in this paper

may find an application in the determination of species of fungi not only in the dermatophytes but in other Fungi Imperfecti, in the Ascomycetes, and in the Basidiomycetes.

Acknowledgment

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EXPLANATION OF PLATES I - III

PLATE I

Microsporon audouini

Fig. 1. Patient C with circular patch on scalp, within which the hairs are infected with *Microsporon audouini.*

Fig. 2. An infected hair which has been in Sabouraud's medium one week. Hyphae have grown out from the spore-sheath into the medium. Magnification, 25.

Fig. 3. *M. audouini* grown for two months on Sabouraud's medium. Natural size.

Fig. 4. Terminal chlamydospores. Magnification, 450.

Fig. 5. Mycelium from patient A showing fusions *f* between the hyphae. Mycelium grown in a hanging drop of Sabouraud's medium, ten days after inoculation. Magnification, 250.

Fig. 6. Hyphal fusions between two mycelia of *M. audouini.* The two hyphae running completely across the field from right to left are from a mycelium derived from patient A, and the four hyphae running from above downwards are from a mycelium derived from patient B. The two mycelia were paired in a hanging drop of Sabouraud's medium one month before the photograph was taken. A fusion between a hypha of the mycelium from patient A and a hypha of the mycelium from patient B is shown at f^1. Magnification, 250.

PLATE II

Microsporon lanosum

Fig. 1. Patient D, infected with *Microsporon lanosum.* The fungus has caused lesions on the eyebrow, glabrous skin, and scalp.

Fig. 2. Infected epidermis (upper left) from patient D, planted in a hanging drop of Sabouraud's medium. Hyphae of *M. lanosum* have grown out into the medium and have given rise to spindles. Magnification, 70.

Fig. 3. *M. lanosum* grown on Sabouraud's medium for five months. There is an abundance of aerial mycelium. Natural size.

Fig. 4. Spindles of *M. lanosum.* Magnification, 450.

Fig. 5. Three hyphal fusions *f f f* in the mycelium of *M. lanosum* obtained from patient D. The mycelium had been growing for one month in a hanging drop of Sabouraud's medium. Magnification, 250.

Fig. 6. Hyphal fusions between two mycelia of *M. lanosum.* The photomicrograph shows parts of two mycelia, one derived from patient D and the other from patient E, which were paired in a hanging drop of Sabouraud's medium. The arrow at f^1 points to a fusion between a hypha of a mycelium derived from patient D and another hypha of a mycelium derived from patient E. Magnification, 250.

PLATE III

Trichophyton gypseum

FIG. 1. Patient G, suffering from sycosis parasitaria (tinea barbae) caused by an infection of *Trichophyton gypseum*. The lesions are in the beard areas, particularly on the upper lip.

FIG. 2. A, a diagrammatic drawing of an infected hair pulled from the beard of patient G, showing the fungal hyphae within the hair and within the follicular sheath: *b*, bulb of hair; *h*, fungal hyphae; *l*, level of skin; *s*, follicular sheath. *B*, a diagrammatic drawing of a fragment of a mycelium of *Trichophyton gypseum* growing on Sabouraud's medium *m*, to show spindles *sp* borne on an aerial hypha. Magnification, 600.

FIG. 3. *T. gypseum* grown on Sabouraud's medium for four months. Natural size.

FIG. 4. Aleuriospores formed on the aerial mycelium of *T. gypseum* growing on Sabouraud's medium in a van-Tieghem cell. Magnification, 250.

FIG. 5. Three hyphal fusions *f f f* in a mycelium of *T. gypseum* obtained from patient G. The mycelium had been growing one month in a hanging drop of Sabouraud's medium. Magnification, 250.

Fig. 6. Hyphal fusions between two mycelia of *T. gypseum*. A mycelium from patient G (of Winnipeg) and a mycelium from patient H (of New York) were paired in a hanging drop of Sabouraud's medium. The wide hypha is from one patient and the three other narrow hyphae are from the other patient. The mycelia had been growing for 13 days: each of the two lines f^1 points to a fusion between a hypha of a mycelium derived from patient G and another hypha of a mycelium derived from patient H. Magnification, 350.

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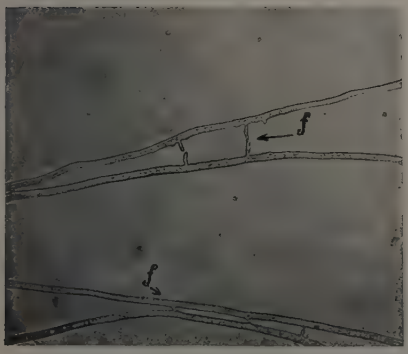

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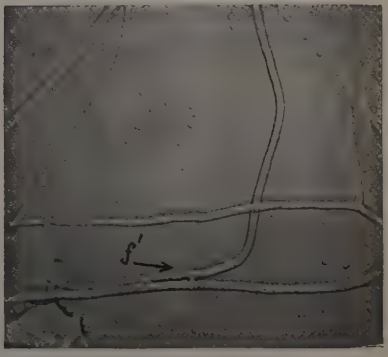

Microsporon audouini

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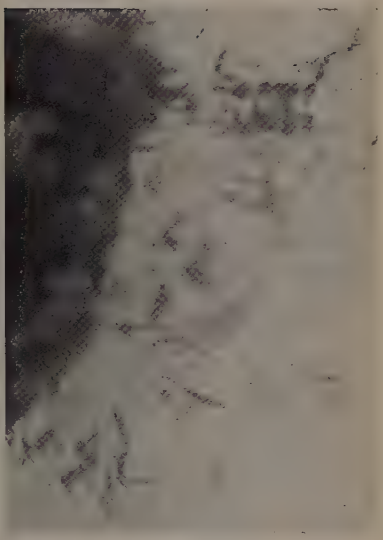

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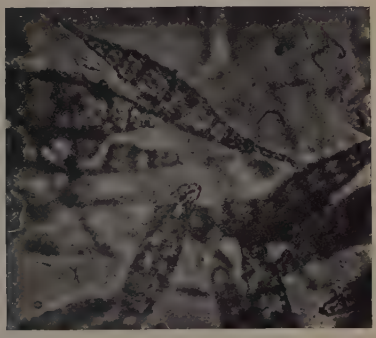

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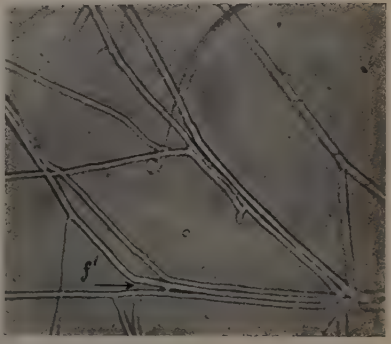

Microsporon lanosum

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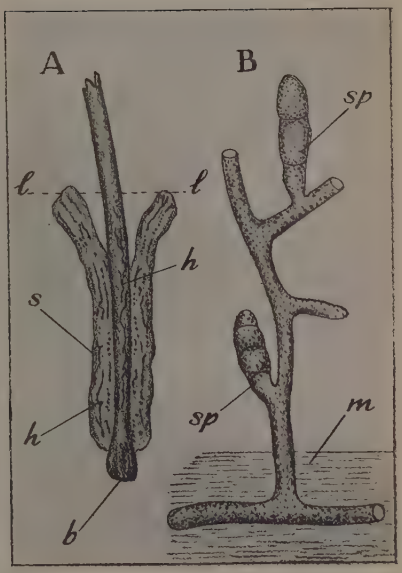

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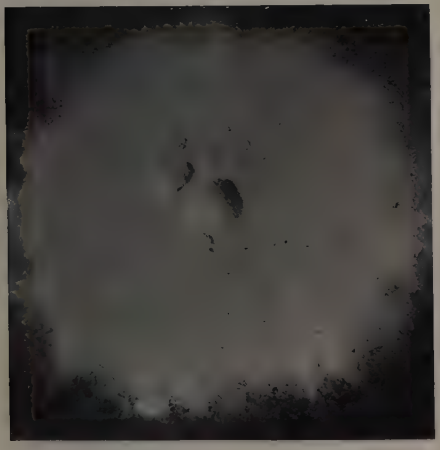

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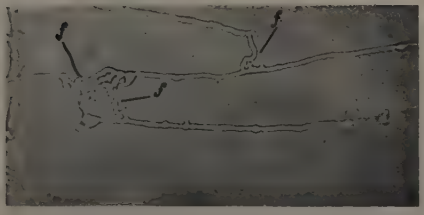

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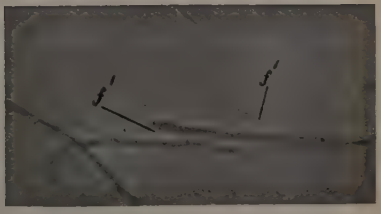

Trichophyton gypseum

PREDICTING THE VALUE OF A CROSS FROM AN F_2 ANALYSIS[1]

By J. B. Harrington[2]

Abstract

The results of extensive breeding work with the cross Marquillo × Marquis were compared with both the original expectation and the expectation calculated from a study of random F_2 populations. The cross was made for the purpose of combining the rust resistance of Marquillo with the many desirable qualities of Marquis. An F_2 population of nearly 40,000 plants was grown in order that there would be a good chance of achieving the desired combination. After five years of breeding effort (nursery, greenhouse and laboratory tests) only six lines remained and none of these were entirely satisfactory. Analysis of random F_2 populations for various important agronomic characters including stem rust reaction indicated that about seven good lines could be expected from 40,000 F_2 plants, providing genetic linkage did not interfere. As this analysis could not include baking quality, a further reduction in the number of selections could be predicted. Results on the best 27 lines from the breeding project showed that baking quality was a difficult character in this cross. The line results also indicated that genetic linkage might be concerned with respect to factors governing rust reaction, seed appearance and crumb color. It was concluded that the F_2 analysis gave a reasonably accurate prediction of the doubtful value of the cross, although it had distinct limitations with respect to characters like baking quality which could not be studied in F_2.

Usually, when a cross is made between carefully chosen parents for a definite breeding purpose, promising plants are selected in F_2 and in several succeeding generations and the cross is carried on until one or more superior new lines have been obtained or until the prospect of obtaining such lines has become very small. However, apparently desirable crosses do not always yield satisfactory results. Not frequently crosses between varieties having well-known desirable characters and no particularly unfavorable characters yield a large preponderance of progeny which could not be considered desirable. Often after a great deal of effort has been put into an attempt to attain a given combination of characters, it is found that the combination has not been fully achieved.

It appears that neither the characters of varieties nor the intensity of the expression of those characters are especially accurate indications of the results to be expected from crossing. In the writer's rust resistance breeding program which has been in progress since 1925 striking examples of good and poor crosses are available. In one case H-44-24*, a new highly rust resistant desirable bread wheat that does not thresh easily, was crossed with Double Cross†, a moderately rust resistant desirable bread wheat, and the F_1 was

[1] *Manuscript received December 2, 1931.*

Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta. The results were reported in full at the meeting of the Associate Committee on Field Crop Diseases at Winnipeg on April 9, 1931.

[2] *Professor of Field Husbandry, University of Saskatchewan.*

* *Produced by Mr. E. S. McFadden, Webster, South Dakota, from the cross Marquis × Yaroslav emmer.*

† *Produced by Dr. H. K. Hayes, University of Minnesota, from the cross (Marquis × Kanred) × (Iumillo × Marquis).*

crossed with Marquis. With comparatively little work 22 very promising resistant lines have been isolated. In contrast to this, the back cross of Marquillo with Marquis has, after six years of effort commencing with a very large F_2 population, resulted in six promising lines none of which is as promising as several of the 22 lines mentioned above.

In order to ascertain the probable economic value of a cross as soon as possible after parent varieties have been selected and the cross made, the writer suggests that a comprehensive study of a random F_2 population be made. This could be done the same season as the main F_2 population is grown or in the previous season, the main sowing being delayed a year. During the past six years a clear-cut illustration of the value of such a procedure has been obtained in the cross Marquillo × Marquis.

Procedure

A cross was made between Marquis and Marquillo in 1925. Marquis and Marquillo are both common bread wheats of normal vigor and fertility with tip awned, fusiform, mid-dense spikes and smooth white glumes. Marquis arose from a cross between Hard Red Calcutta, an Indian wheat, and Red Fife, which probably came originally from Russia. Marquillo (3) resulted from a cross between Marquis and Iumillo, a durum wheat of good yield, fair quality and extremely high rust resistance. Table I gives a comparison of Marquis and Marquillo for various agronomic characters of importance.

TABLE I

COMPARISON OF MARQUIS AND MARQUILLO FOR IMPORTANT AGRONOMIC CHARACTERS AS OBTAINED FROM PLOT AND LABORATORY TESTS COVERING SIX YEARS

Character	Marquis	Marquillo	Character	Marquis	Marquillo
Height in inches	42	37	Grain yield in bushels per acre	38.5	41.6
Days seeding to ripe	111	109	Grains weight per bushel	65.3	64.3
Straw strength in per cent	88	94	Seed appearance rating	100	*80*
Non-shattering in per cent	97	98	1000 kernel weight in gm.	30.6	33.0
Resistance to stem rust	*poor*	good	Milling quality score	95.8	95.1
Resistance to leaf rust	good	good	Total baking score	97	96
Resistance to bunt	fair	fair	Crumb color score	9.0	7.8
Resistance to root rot	fair	fair			

In 1926 and again in 1930 random F_2 populations of the cross Marquillo × Marquis were studied. In 1926 plant height, earliness of maturity and rust resistance were studied. During the succeeding five years, this cross was handled as an extensive breeding investigation involving nursery, greenhouse and laboratory tests and a total F_2 population of 36,800 plants was reduced to six promising lines. Owing to the difficulty of obtaining satisfactory combinations of characters in this cross, and owing to the disappointing fact that no line was obtained which fulfilled the original expectation of a combination of

Marquillo rust resistance with the milling and baking quality of Marquis, a special study of a population of several hundred F_2 plants was made in 1930. This was made possible through the keeping of a substantial reserve of F_2 seed from 1926.

In this study an analysis was made for the characters plant height, grain yield, seed appearance and rust reaction. Interrelationships of these characters were also investigated. These results, together with those of the 1926 studies, formed excellent material with which to compare the data obtained during 1929 and 1930 on the best 27 lines.

F_2 Results

Rust Reaction

A study was made of the reaction of 781 Marquillo × Marquis F_3 families in the seedling stage in the greenhouse at an average temperature of 69.6° F. to physiologic form 21 of *Puccinia graminis tritici*. Form 21 was used in the greenhouse tests because of its prominence in Western Canada and because Marquis and Marquillo react quite differently to it, Marquis being susceptible and Marquillo resistant. Only eleven, or almost exactly one sixty-fourth of the families were resistant, indicating the operation of three main genetic factors. The results are shown in Table II.

TABLE II

DISTRIBUTION OF 781 F_2 PLANTS* OF THE CROSS MARQUILLO × MARQUIS, ACCORDING TO THE REACTION OF THEIR F_3 SEEDLING PROGENY TO FORM 21 IN THE GREENHOUSE AT A TEMPERATURE OF APPROXIMATELY 70° F.

Material	Distribution of F_2 plants according to the classes determined by the reaction of F_3 seedlings					Number of F_3 families
	R**	HR	H & I	HS	S	
Marquis					15	
Marquillo	13					
F_2	11	29	91	261	389	781

* *This was not a complete random sample with respect to rust reaction, since approximately 50 of the most susceptible plants were discarded in the field. Even with these 50 added the fit to a three factor hypothesis remains quite good.*

***R, resistant; HR, heterozygous resistant; H, heterozygous; I, intermediate; HS, heterozygous susceptible; S, susceptible.*

Field nursery results in 1926 and 1927 indicated that three or more factors controlled the reaction to the forms of rust present. Tests from various field rust cultures showed the presence of only form 21 in the 1926 nursery, but in the epidemic year 1927 there were present forms 15, 17, 21, 29, 30 and 36. Form 21 appeared to be the most abundant of these.

Both greenhouse tests to form 21 and the field tests indicated the existence of three main genetic factors for rust reaction. The value of these results was increased by finding that there was a strong positive relationship between the

seedling and the field reaction†. This information was obtained from a study of 129 F_3 families with respect to the correlation between their seedling reactions to form 21 and their field reactions in 1927 to a mixture of forms. The coefficient of contingency was 0.69 ± 0.062.

In December 1930 several hundred F_3 families of the F_2 population grown during the preceding summer were tested to form 21 in the seedling stage. The results agreed well with the previous results in indicating three genetic factors governing the reaction.

From the breeding standpoint the significant feature of these findings was that only about 1.5% of the F_2 plants resembled the Marquillo parent variety in rust reaction.

TABLE III

DISTRIBUTION OF MARQUILLO × MARQUIS HYBRIDS FOR DAYS FROM SEEDING TO MATURITY

Material	Class centres for days from seeding to maturity							Av. no. days, seeding to ripe
	50	53	56	59	62	65	68	
Marquis		13	94	49	16	6		57.4±2.33
Marquillo	6	91	61	8				54.3±0.94
F_2 plants	7	55	231	62	23	10	3	56.6

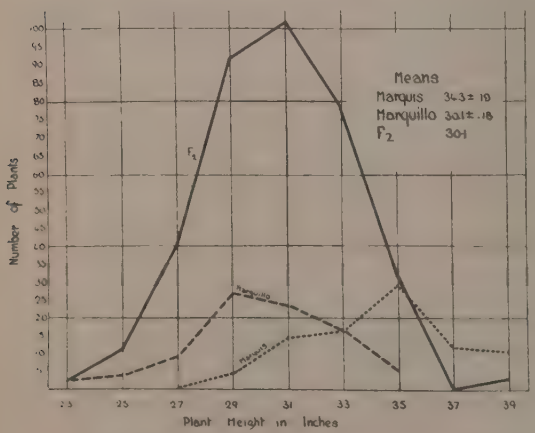

FIG. 1. *Marquillo × Marquis plant height, 1930.*

Earliness of Maturity

Marquillo is about two days earlier in maturity than Marquis, as shown in Table I. The F_2 population showed no definite indication of dominance (see Table III). In this cross an attempt to obtain segregates with the earliness of Marquillo might mean the loss of a fairly large proportion of the hybrids. Therefore, since a two day difference in time of maturity is not especially important, there would seem to be no good reason to discard any of the hybrids on this basis.

Height of Plant

Height of plant is important where short straw frequently occurs in dry seasons and the binder is the usual harvesting machine. Marquillo is several inches shorter than Marquis. The F_2 results in 1926 as well as in 1930 indicated that this character was probably dependent on several genetic factors. There was no clear-cut indication of dominance, but the F_2 in the 1930 study had

† *Previously Harrington and Smith (2) had noted this relationship.*

the same average height as Marquillo (see Table IV). Only 35 F_2 plants equalled the average height of Marquis and more than two-thirds of the F_2 plants were three inches or more shorter than Marquis. The results on height are shown graphically in Fig. 1. It would seem to be desirable to discard the shortest plants to the extent of about a third of the population. The chances remained that most of the retained hybrids would not excel Marquillo in height. On the other hand, the height of Marquillo could hardly be considered a sufficiently important disadvantage to warrant discarding much more than the plants below the main Marquis range in height.

TABLE IV

PLANT HEIGHT OF MARQUILLO × MARQUIS HYBRIDS, 1930

Material	Distribution of plants according to their height in inches									Average plant height
	23	25	27	29	31	33	35	37	39	
Marquis			1	4	15	17	29	13	11	34.8±0.19
Marquillo	2	4	8	26	23	17	5			30.6±0.18
F_2	7	11	40	90	102	78	32	1	2	30.6

Seed Characters

Seed character refers principally to milling and baking quality, shape, plumpness, color (lustre) and size. These characters are particularly important where a conservative, exacting foreign market is to be suited. Unfortunately, it is not possible to obtain milling and baking data on single plants. As for the other characters, it is possible by careful laboratory inspection to combine in a single expression the value of a line with respect to all of them. This was done in the 1930 F_2 study under the term "seed appearance." The results appear in Table V and are shown graphically in Fig. 2. It is apparent that there is a decided preponderance of the seed characters of Marquillo in the F_2. Only six of the 356 F_2 plants equalled the Marquis mean in seed appearance. It would seem necessary therefore to discard a very large proportion of the hybrids in order to retain the seed appearance of Marquis.

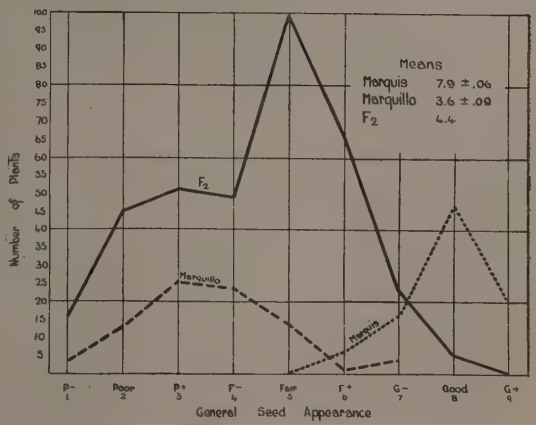

FIG. 2. *Marquillo × Marquis seed appearance, 1930.*

Grain Yield

Grain yield is generally considered one of the most important characters in a cereal crop. Unlike height of plant, earliness of maturity and other

TABLE V

SEED APPEARANCE OF MARQUILLO × MARQUIS HYBRIDS, 1930

Material	Distribution of plants according to their seed appearance taken on the basis of shape, color and plumpness									Average seed appearance
	P− 1	Poor 2	P+ 3	F− 4	Fair 5	F+ 6	G− 7	Good 8	G+ 9	
Marquis					1	6	16	47	20	7.9± .06
Marquillo	3	13	26	24	14	2	3			3.6± .09
F_2	16	45	52	49	99	66	23	5	1	4.4

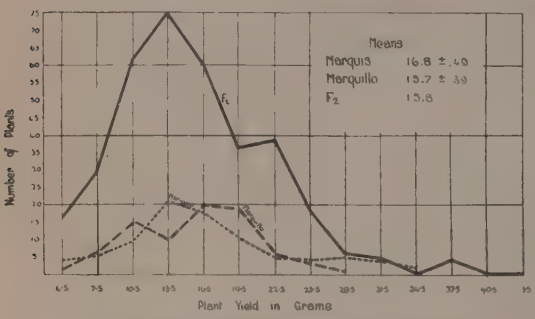

FIG. 3. *Marquillo × Marquis grain yield, 1930.*

characters discussed, grain yield is not usually obtained on hybrid lines until sufficient seed is available for a proper rod row replicated plot test. This is usually not until F_4 at the earliest and frequently not until F_5 or F_6. To obtain a reliable index of yielding ability in a given cross and especially to obtain relationships between yield and other important characters before the cross had progressed beyond the first segregating generation would be highly desirable. This may be accomplished with F_2 plants. The actual yield of any given plant tells very little respecting the inherited yielding ability of that plant, but the actual yields of all the individual plants in a population give a reliable estimate of the yielding ability of the cross as a whole.

TABLE VI

DISTRIBUTION OF MARQUILLO × MARQUIS HYBRIDS FOR GRAIN YIELD, 1930

Material	Class centres for grain yield in grams per plant							Average plant yield
	6	12	18	24	30	36	42	
Marquis	10	30	30	9	9	2		16.8±0.49
Marquillo	9	25	39	10	2			15.7±0.39
F_2	47	137	97	56	12	5	2	15.8

The F_2 results on grain yield are summarized in Table VI. The parent varieties did not differ significantly in yield. The apparent higher yield of Marquis is in fact opposite to the usual plot results where Marquillo generally yields higher, as shown in Table I. The F_2 curve for yield as shown in Fig. 3 closely approaches those of the parent varieties, indicating that no trouble should be anticipated with respect to that character.

Analysis of the F_2 Results for the Estimation of the Probable Success of the Cross

The F_2 results may be used in predicting the probable possibility of obtaining hybrid lines combining the desirable characters of the parent varieties as far as the characters studied are concerned. The rust reaction results showed that about one sixty-fourth of the hybrids would probably be homozygous for Marquillo resistance. The results on earliness indicated that no attempt to select for earlier maturity than that of Marquis would be advisable as the sacrifice of population would hardly be justified by the additional earliness. The results on plant height indicated the desirability of discarding the shortest one-third of the population. The seed appearance results showed that the chances of having Marquis appearance in selected segregates were small, for only one-sixtieth of the F_2 plants equalled the Marquis mean. Results on grain yield did not show the necessity of discarding any hybrids.

The foregoing analysis may be summarized numerically in order to obtain a figure representing the proportion of F_2 plants that would probably be satisfactory for the characters studied. The calculation is as follows:— $\frac{1}{64} \times \frac{2}{3} \times \frac{1}{60} = \frac{1}{5760}$. That is, one plant in every 5760, or approximately 7 plants from a population of 36,800 (the actual F_2 population) should be satisfactory for the characters studied providing the factors for each character were independently inherited. Just how much these 7 individuals would be reduced in number on the basis of milling and baking quality or any other character which could not be studied, remains unknown.

Results on the Best Hybrid Lines Arising from the Breeding Project

An extensive breeding project was commenced in 1925 with the cross Marquillo × Marquis for the purpose of combining the desirable characters of these two varieties. As seen from the description of Marquillo and Marquis in Table I, the goal would not appear to be particularly difficult to attain.

TABLE VII

REDUCTION OF THE NUMBER OF MARQUILLO × MARQUIS LINES DURING THE FIRST SEVEN GENERATIONS

Number of lines		Generation	Year	Basis of elimination
Grown	Discarded			
36,800	32,600	F_2	1926	Field rust reactions, height, earliness, vigor.
4,200	3,781	F_2	1926–7	Seedling reaction of F_3 progenies to form 21.
519	417	F_3	1927	Field rust reaction, also earliness, etc.
102	19	F_4	1928	Preliminary replicated plot test for yield, etc. milling and baking test.
83	56	F_5	1929	Replicated plot tests, root rot tests, milling and baking tests.
27	21	F_6	1930	Same as in 1929 and smut tests
6		F_7	1931	Same as in 1930

Nevertheless, in order to be reasonably sure of satisfactory results, a large F_2 population was used. During the course of six years of nursery, greenhouse and laboratory tests, this population was reduced to six good lines, none of which fulfilled the original expectations. The reduction in the number of lines from year to year is shown in Table VII.

As shown in Table VII, there were 83 lines remaining under test in 1929. Fifty-six of these were discarded in the fall on account of low yield and insufficient rust resistance. In the following summarization of results for the remaining 27 lines data from replicated rod row tests covering three years have been used. It will be seen that the practical value of lines has been considered and the theoretically desirable elimination of any hybrid not measuring up fully to the best characters of both parent varieties has not been rigidly practiced.

TABLE VIII

DISTRIBUTION OF THE 27 LINES ACCORDING TO THEIR RUST REACTION ON THE BASIS OF THE RESISTANCE OF MARQUILLO*

More R	As R	10% less R	20% less R	30% less R	40% less R	50% less R	60% less R	70% less R
1	2	2	4	5	2	5	4	1

* *Marquis was approximately 300% less resistant than Marquillo.*

Rust Reaction

Seedling tests to pure cultures of form 21 have shown the 27 best F_6 lines to be fairly rust resistant with a range extending from the resistance of Marquillo to a much lesser amount, and averaging distinctly less resistant than Marquillo. Only three of the 27 best lines were as resistant as Marquillo. The results of nursery tests in 1930 on all of the 27 lines at Indian Head, Grenfell and Carmichael, of part of them at Winnipeg, St. Paul and Fargo, and of part of them at Saskatoon in 1928 are summarized in Table VIII.

TABLE IX

DISTRIBUTION OF THE 27 LINES FOR TIME FROM SEEDING TO MATURITY IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930 RESULTS)

Material	98	99	100
Marquis			×
Marquillo	×		
Hybrid lines	9	15	3

Earliness of Maturity

No selection was made upon the basis of earliness of maturity as both parent varieties mature moderately early, consequently, the line results would be expected to agree fairly well with those from the F_2 study. Table IX, giving the summarized data, shows this to be the case.

Height of Plant

In the first segregating generation each field resistant plant was measured for height when harvested and only those at least 95% as tall as the nearest Marquis check plots were kept. Approximately one-third of the population

was discarded upon this basis. However, the F_2 analysis indicated that very few plants would be likely to yield lines resembling Marquis in height. The actual line results show, as summarized in Table X, that none of the lines equalled Marquis in height. But the general average for the lines was higher than that of the random F_2 population, as was expected.

TABLE X

DISTRIBUTION OF THE 27 LINES FOR HEIGHT OF PLANT IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930)

	85.5	87.5	89.5	91.5	93.5	95.5	97.5	99.5
Marquis								×
Marquillo					×			
Hybrid lines	1		3	3	9	9	2	

Seed Characters

In addition to a combined expression representing for each line its seed character as explained in connection with the F_2, separate notes were taken on the lines for bushel weight, seed plumpness and seed color. The summarized results are shown in Tables XI to XIV. In bushel weight and seed plumpness the lines averaged closer to Marquillo than to Marquis, but a fair proportion of hybrids in each case were about as good as Marquis. For seed color (lustre) the poor appearance of Marquillo had a very dominating influence and only one of the 27 lines was like Marquis.

TABLE XI

DISTRIBUTION OF THE 27 LINES FOR WEIGHT PER MEASURED BUSHEL IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930 RESULTS FROM REPLICATED ROD ROW PLOTS)

	95	96	97	98	99	100	101
Marquis						×	
Marquillo			×				
Hybrid lines	2	4	6	9	4	1	1

TABLE XII

DISTRIBUTION OF THE 27 LINES FOR SEED PLUMPNESS IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930 RESULTS FROM REPLICATED PLOT TESTS)

	Class centres for seed plumpness						
	88	91	94	97	100	103	106
Marquis					×		
Marquillo				×			
Hybrid lines	1	3	5	9	6	2	1

The combination character "seed appearance" expresses best the apparent value of a variety as seen from visual examination of a handful of seed. The data on this character (see Table XIV) resemble closely those of the F_2 in

Table V, excepting that the line data show an absence of lines poorer than Marquillo. This difference is due to the fact that each year lines with very poor seed appearance were discarded.

TABLE XIII

DISTRIBUTION OF THE 27 LINES FOR SEED COLOR (LUSTRE) IN PERCENTAGE OF MARQUIS (RESULTS FROM 1930 QUADRUPLICATE PLOT TESTS)

	80	84	88	92	96	100
Marquis						×
Marquillo		×				
Hybrid lines		5	8	8	5	1

TABLE XIV

DISTRIBUTION OF THE 27 LINES FOR GENERAL SEED APPEARANCE IN PERCENTAGE OF MARQUIS (RESULTS FROM THE QUADRUPLICATE ROD ROW PLOT TEST IN 1930)

	80	84	88	92	96	100
Marquis						×
Marquillo	×					
Hybrid lines	2	4	10	9	1	1

TABLE XV

DISTRIBUTION OF THE 27 LINES FOR GRAIN YIELD IN PERCENTAGE OF MARQUIS (RESULTS FROM THE QUADRUPLICATE ROD ROW PLOT TESTS IN 1929 AND 1930)

	Class centres for mean yields of lines					
	81	86	91	96	101	106
Marquis					×	
Marquillo						×
Hybrid lines	1	2	7	10	5	2

Grain Yield

The line results on grain yield were very surprising, for, as shown in Table XV, the average yield of the lines is considerably below that of the parent varieties, whereas in the F_2 results the mean was intermediate with respect to the parent variety means. The expectation was that nearly all the lines would be high in yield, since both parent varieties are high and since the general tendency throughout the selection in the main segregating generations (F_2, F_3 and F_4) would be to eliminate lines that appeared to be distinctly inferior in yielding capacity. Genetic linkage seems to be involved.

Milling and Baking Quality

Both Marquillo and Marquis mill well and in the eyes of Minnesota millers, they are almost equally desirable in baking quality. The chief difference between these varieties is that flour from Marquillo has much more carotin pigment than Marquis flour. However, the millers find that the slightly yellowish color is taken out as far as is necessary by bleaching. The distribution of the hybrid lines with respect to crumb color is given in Table XVI. The fact that most of the lines showed distinct inferiority to both parent varieties in 1930 is inexplicable.

Marquis and Marquillo were closely alike in computed baking score where bleached flour is used. As most millers regularly bleach flour, the results on the lines are presented in Table XVII on that basis. The results were even more striking and inexplicable than in the case of crumb color. In 1929 no

TABLE XVI

DISTRIBUTION OF THE 27 LINES FOR CRUMB COLOR IN PERCENTAGE OF MARQUIS AS OBTAINED FROM AVERAGING THE RESULTS FROM THE "BROMATE" AND THE "BLEND-BROMATE" BAKING METHODS USED ON BLEACHED FLOUR

	75-78	79-82	83-86	87-90	91-94	95-98	99-102	103-106	107-110
Marquis							1928-30		
Marquillo			1930		1928	1929			
Hybrid lines 1928*									4
Hybrid lines 1929			4	12	3	5	2		
Hybrid lines 1930*	2	1	3	2					

* *Many of the lines were not tested.*

line averaged as high a score as the parent varieties and nearly a third of them were distinctly low, whereas in 1928 and in 1930 all the lines tested gave good results.

TABLE XVII

DISTRIBUTION OF THE 27 LINES FOR COMPUTED BAKING SCORE IN PERCENTAGE OF MARQUIS AS OBTAINED FROM AVERAGING THE RESULTS FROM THE "BROMATE" AND THE "BLEND-BROMATE" BAKING METHODS USED ON BLEACHED FLOUR

	79-82	83-86	87-90	91-94	95-98	99-102	103-106	107-110
Marquis						×		
Marquillo					1928	{1929 1930		
Hybrid lines 1928*						2	1	1
Hybrid lines 1929	2	5	11	6	2			
Hybrid lines 1930					3	4		1

* *Many of the 27 lines were not tested.*

Reaction to Root-rotting Organisms

The Marquillo × Marquis lines and the parent varieties were tested for their reaction to some common root-rotting organisms in a special disease garden and in the greenhouse in 1929, and in the disease garden and at Grenfell, Carmichael and Humboldt in 1930. The 1929 disease garden test was unsatisfactory due to droughty conditions. The greenhouse test was made at the seedling stage to virulent strains of *Helminthosporium sativum*, *Fusarium culmorum* and *Ophiobolus graminis*. The results were not particularly striking. The parent varieties appeared to be about equally subject to seedling infection excepting that Marquillo seemed slightly more susceptible to *F. culmorum* than Marquis. None of the lines showed higher infection than the parent varieties.

The 1930 tests at the three points outside Saskatoon gave unsatisfactory results, due to the dry season. The results from the disease garden are summarized in Tables XVIII and XIX. No significant difference in reaction was found with respect to the two varieties and their hybrids either for lesioning of the crown or for number of blighted plants.

TABLE XVIII

DISTRIBUTION OF MARQUILLO × MARQUIS HYBRID LINES AND MARQUILLO WITH RESPECT TO THEIR DIFFERENCE FROM THE NEAREST MARQUIS CHECKS IN AVERAGE INDEX OF LESIONING

Cultures	Class centres for differences from Marquis checks in index of lesioning (− = less lesioning, + = more)							
	−12	−9	−6	−3	0	+3	+6	+9
Marquillo	1			3	1	1		1
Hybrid lines	1	3	8	6	14	9	1	

TABLE XIX

DISTRIBUTION OF MARQUILLO × MARQUIS HYBRID LINES AND THE PARENT VARIETIES WITH RESPECT TO THEIR DIFFERENCE FROM THE NEAREST MARQUIS CHECKS IN TOTAL NUMBER OF BLIGHTED PLANTS

Cultures	Class centres for differences from Marquis checks in number of blighted plants						
	−21	−14	−7	0	+7	+14	+21
Marquillo			2		3	1	1
Hybrid lines	3	7	5	9	8	7	3

As Marquis does not suffer greatly from root rot under ordinary field conditions in Western Canada, the hybrid lines may be considered reasonably satisfactory as far as the results of these tests are concerned.

Reaction to Covered Smut

The parent varieties and hybrid lines were tested in duplicate plots for bunt reaction in 1930. The average percentages of plants not infected were as follows:

Marquis, 33 ± 1.7. Marquillo, 32 ± 1.7. Hybrid lines, 35.

Marquis and Marquillo were not significantly different for bunt reaction and the hybrid lines averaged about the same as the parent varieties. Marquis under ordinary field conditions is moderately susceptible to bunt.

Relationships Between Characters

The interrelationships of plant height, seed appearance and grain yield were studied in the F_2 data. The following correlation coefficients were obtained:

Grain yield and plant height.....0.57 ± 0.024
Grain yield and seed appearance.....0.25 ± 0.034
Plant height and seed appearance.....0.47 ± 0.028

These values show distinct relationships, but as they could easily be largely the result of environmental influences, similar interrelationships were worked

out for the data on the Marquis parent plants of which 90 were studied. The coefficients are as follows:

Grain yield and plant height	0.78 ± 0.028
Grain yield and seed appearance	0.50 ± 0.053
Plant height and seed appearance	0.59 ± 0.046

The Marquis results show relationships fully as high as those of the F_2, consequently it cannot be said that the latter indicate genetic linkage.

In addition the relationship between the yields of F_2 plants and the seedling reaction of their F_3 progenies to stem rust form 21 was obtained; r was 0.08 ± 0.050. Similarly for plant height and rust reaction the value of r was 0.10 ± 0.047.

Interrelationships were then worked out for the results on the 27 best lines. The correlations were as follows:

Grain yield and computed baking score	0.2 ± 0.13
Grain yield and rust resistance*	0.1 ± 0.13
Grain yield and seed plumpness	0.4 ± 0.11
Grain yield and crumb color	0.2 ± 0.13
Grain yield and seed appearance	0.1 ± 0.13
Crumb color and seed appearance	0.6 ± 0.09
Rust resistance and seed appearance*	0.3 ± 0.12
Crumb color and rust resistance*	0.3 ± 0.12
Plant height and rust resistance	0.1 ± 0.13
Plant height and computed baking score	0.2 ± 0.13

There were only two relationships that appeared to be significant. One concerns seed plumpness and grain yield and probably has no genetical significance since such a relationship would be expected as a result of environmental effects. The other relationship is between crumb color and seed appearance and may have some genetical importance. It is apparent that the study of character interrelationships does not explain the line results shown in Tables XIV, XV and XVI.

Discussion

Every year scores of new crosses are made in the small grains at the various experimental stations scattered across the country and many of these crosses never result in worthwhile new varieties. It may be assumed that most of these crosses are made after careful thought had been given to the choice of parent varieties. The conclusion is then obvious that apparently good combinations of varieties do not always prove satisfactory. This being the case, the sooner critical tests can be made of the probable practical value of the crosses, the greater will be the amount of time and energy available for

* *The usual physical relationships between rust reaction and other characters did not affect this correlation as the data on yield, seed appearance and crumb color were secured at Saskatoon as summarized in Tables XIV, XV and XVI, whereas the rust data were averaged from 1930 tests in a number of localities.*

use upon those that are really promising. The F_2, or first segregating generation, offers the earliest opportunity for such critical tests. The present study illustrates the use and value of an F_2 study for this purpose.

The desirability of a cross as far as disease resistance is concerned, depends not only upon the degree of resistance of the resistant parent variety but also upon the frequency with which desirable resistance occurs in the hybrids and upon relationships between resistance and undesired characters. Marquillo has sufficient stem rust resistance to insure a farmer a reasonable degree of security against heavy loss from rust. The substitution of Marquillo for the older rust susceptible varieties, such as Marquis, has been going on for several years in Minnesota and testifies to the practical value of the new variety. However, when an attempt was made to improve upon Marquillo by backcrossing it with Marquis, one of the greatest obstacles was the paucity of resistant progeny.

The F_2 results showed that only about one sixty-fourth of the hybrid plants possessed resistance as high as that of Marquillo. All of the rest of the plants were more or less susceptible. Most of these would have yielded some resistant plants upon segregation of their progeny but to await these further generations for the selection of more resistant plants would have taken valuable time and increased the cost and complexity of the work. Consequently it was considered that approximately 98.5% of the F_2 population was of little value and warranted discarding. This was done but the loss was heavy since, for the sake of one character, 98.5% of the work of crossing and of growing, examining and testing the F_1 and the F_2 was lost. The evidence furnished later by the line results in F_4, F_5 and F_6 demonstrated that such an initial loss is a severe handicap for it reduces the population so greatly that insufficient material is likely to be left to furnish the desirable combinations of characters for which the cross was made. This line evidence indicated that a very heavy elimination of progeny for a single unfavorable character at the outset of a cross would probably warrant dropping the cross without going further, especially if a number of other important characters were still to be considered.

For yield and earliness, both parent varieties were satisfactory and the F_2 distributions, as expected, resembled the distributions for the parent varieties fairly closely. There appeared to be no reason for discarding hybrids on the basis of these characters. The F_6 line results showed that the F_2 analysis correctly predicted no difficulty as to earliness.

However, the results on the best 27 lines remaining after five years of breeding showed 20 of these lines to be inferior to both parent varieties in yielding ability. This was not expected, as both parent varieties yielded well and Marquillo, the rust resistant variety, excelled Marquis, the susceptible variety, in this respect. The line results suggest genetic linkage between factors for rust susceptibility and factors for yielding capacity. This was not proved however. The lines varied from one that was more resistant than Marquillo to one that was 70% less resistant than that variety, yet no correlation indicating genetic linkage was found between rust reaction and yield. Furthermore no indication of genetic relationship was discovered

between grain yield and rust reaction in the F_2 study. This was as expected since the inheritance of rust reaction is complex and that of yielding ability has been found in other crosses to be complex as shown by the studies of Waldron (4), Bridgford and Hayes (1) and others. It may be concluded that the F_2 results on yield were somewhat misleading.

For height of plant, the F_2 distribution was practically identical with that of Marquillo. This indicated that the hybrid lines would on the whole resemble Marquillo in height. Discarding the shortest hybrid plants to the extent of 35% would remove most of any short plants resulting from possible transgressive segregation. This would result in the 27 selected lines averaging slightly taller than Marquillo. The results in Table X accord with this supposition. Therefore, the F_2 results on plant height correctly intimated what was to be expected from the selected lines.

Seed characters are extremely important, particularly grain plumpness, color and quality. F_2 results fail to predict milling and baking quality as no reliable tests have yet been devised for detecting such qualities in the small amount of seed obtainable from a single plant. In the cross Marquillo × Marquis, no great difficulty as to quality was anticipated owing to the desirability of both parent varieties in this regard. It is true that Marquillo is less desirable than Marquis in flour color and, on that account, in general baking score, but the writer understands* that United States millers and bakers who are accustomed to handling Marquillo, like it about as well as Marquis. In protein content, water absorption and loaf volume, Marquillo flour ranks almost equal to that of Marquis. The line results were a great surprise for they showed the lines to have poorer quality than Marquis in both 1929 and 1930 although better quality in 1928. The selected lines averaged only slightly higher in quality than Marquillo. If a milling and baking test on the F_2 plants had been possible, the latter results on the lines might have been predicted. This would have been a further reason for considering the cross undesirable to continue.

F_2 results may be used to estimate the possibilities of the cross as far as morphological seed characters are concerned. A general note was taken on the seed of each plant for general appearance which included plumpness, color, shape and size. Only 6 of the 356 F_2 plants equalled the Marquis mean in seed appearance Here again, as with rust reaction, it would seem necessary to discard over 98% of all the hybrids if only those equalling Marquis in seed character were to be saved. If this test for seed appearance had been made in 1926 instead of in 1930, it is probable that the results added to those obtained on rust and plant height would have been considered sufficient to justify discarding the cross without carrying it further. However, a critical study of F_2 for seed appearance was not made at the beginning of the investigation, but the lines that were poor in seed appearance were discarded during the first four generations. Of the best 27 lines, only one equalled Marquis in

* *From a statement by Dr. R. K. Larmour regarding the high regard for Marquillo expressed by Minneapolis millers at a recent conference of cereal chemists.*

seed appearance. The result agrees very well with the F_2 analysis and reveals again the value that would have existed in a study of an experimental F_2 before the breeding project was carried forward in a large way.

Summing up, the results of the present study indicate that a preliminary experimental F_2 population of several hundred plants should be analyzed for all important characters at or before the commencement of extensive work on a cross. In the Marquillo × Marquis cross the F_2 was quite satisfactorily used for a thorough individual plant study of stem rust reaction, plant height, earliness of maturity, yield of grain and various seed characters. Other characters could also have been used, including tillering, protein content (where the amount of seed per plant is plentiful) resistance to frost, weathering and sprouting, reaction to leaf rust, root rots, smuts, hot winds, etc. In the present study, part of the F_2 analysis was made on a specially grown random population several years after the commencement of the cross. This later F_2 study was made primarily to explain why none of the best F_6 lines combined all of the favorable characters of the two parent varieties as was desired. The analysis of the two F_2 populations and the linking up of the results with those from the best F_6 lines has shown definitely how advantageous a complete F_2 study would have been in the beginning.

As was stated earlier in this paper, the Marquillo × Marquis cross, while it has produced several meritorious lines, each of which appears to excel Marquillo distinctly in general value, did not fulfil the writer's original expectations and has been much less worthwhile than other crosses, among which may be mentioned the (H-44-24 × Double Cross) F_1 × Marquis cross. Undoubtedly among the crosses made each year in Canada there are many corresponding in comparative value to the two crosses above mentioned. It would therefore seem highly desirable to make an F_2 analysis as outlined here and then discard the cross immediately if the chances for success without great effort and cost are not reasonably large. Where such analysis convinces the investigator that the cross is worth continuing, it is very probable that the data accumulated in the F_2 study will be a valuable aid in the annual selection of desirable plants. As a matter of fact, in most crosses, an analysis of some characters is usually made in F_2 for the purpose of assisting the selection work.

While the advantages of a preliminary F_2 analysis are large, it should be remembered that an F_2 distribution may sometimes be misleading. Furthermore, genetic linkage may be present as a distinct obstacle to the accomplishment of the breeding purpose. Ascertaining the relations between various characters of the F_2 is therefore advisable.

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A COMPARISON OF GLUTENIN AND GLIADIN PREPARED FROM ONE FLOUR BY VARIOUS METHODS[1]

By R. K. Larmour[2] and H. R. Sallans[3]

Abstract

Gliadin and glutenin were prepared from gluten of hard red spring wheat flour by five different methods and analyzed by the Van Slyke procedure. The gliadin preparations gave very similar results, indicating that differences in manipulation have little effect on this protein. The glutenin preparations showed very great differences in nitrogen distribution, the greatest being in ammonia nitrogen and in basic nitrogen. There is considerable evidence that these two nitrogen fractions tend to be inversely proportional. This is particularly applicable to preparations made by methods involving use of alkaline solutions. Glutenin isolated by use of Blish and Sandstedt's acetic acid method, using 0.007 *N* acid appeared to be purer than the other preparations and contained the highest percentage of ammonia nitrogen. This protein, when dissolved in 0.025 *N* sodium hydroxide solution and allowed to stand for one week, lost 4.8% of its nitrogen in the form of ammonia, but the basic fraction was unchanged. It seems likely that any method for isolating glutenin involving use of alkaline solutions would involve loss of nitrogen in the course of the preparation and the nitrogen distribution obtained by the Van Slyke analysis would therefore be in error. The use of very dilute acetic acid is recommended but exposure to even dilute solutions of the strong alkalies should be rigorously avoided in the preparation of glutenin.

Since the demonstration of their existence, the gluten proteins, gliadin and glutenin, have appealed to investigators as the constituents of flour most likely to influence the baking strength. This is a logical conclusion, as from the nature of crude gluten one would expect it to be responsible for the elasticity and gas-retaining properties of the dough. As these two proteins comprise approximately 90% of the total flour protein, there have been numerous attempts to discover a direct relationship between protein content of wheat and flour with the strength as determined by the baking test. A high degree of correlation between these variables would warrant the use of protein, as calculated from the Kjeldhal nitrogen, in estimating the commercial worth of any particular sample. The investigations made by Zinn (21), Mangels (19), and Hayes, Immer and Bailey (9), show that a relationship undoubtedly exists, but that the quantity of protein alone is not an adequate measure of strength. The variability not accountable to variation in amount of protein has been generally attributed to another factor, namely, the quality of the protein. Although Larmour (17, 18) has shown that by use of the Werner bromate formula for experimental baking much of this unaccountable variation is eliminated, there still remain differences between wheat flours that cannot be wholly related to the quantity of protein present. This variability may be

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attributable to qualitative differences or to manipulative error, principally in the baking test, or to both causes.

If qualitative differences do exist they may be attributable in part to variation in either chemical or colloidal properties of the proteins. Not a great deal of comparative work on chemical composition of these proteins has been done. Blish (1) prepared and analyzed glutenin and gliadin from both a weak and a strong flour but was unable to find any significant differences in the various nitrogen fractions as determined by the Van Slyke procedure. Cross and Swain (5) carried out a similar study using flour milled from four varieties of wheat and came to the conclusion that neither the gliadins nor the glutenins from the various flours could be chemically differentiated. Hoffman and Gortner (10) concluded "that the gliadin prepared from different varieties of wheat, including wheats which give strong and weak flours, are identical . . . . This identity apparently holds for physical as well as chemical properties." Without any very extensive work on the subject, there seems to have arisen the general belief that proteins from weak and strong flours cannot be differentiated by means of the Van Slyke distribution. However, when one compares the results of analyses reported by different investigators, very wide ranges in values of many of the nitrogen fractions are noted. Thus, in data collected from the literature by Larmour (16), variations are found as shown in Table I.

TABLE I

RANGE OF VARIATION OF NITROGEN FRACTIONS OF GLIADIN AND GLUTENIN PREPARED FROM WHEAT (*T. vulgare*) FLOUR. (TAKEN FROM TABLES III AND IV LARMOUR (1928))

Nitrogen fraction	Range of values	
	Gliadin	Glutenin
Ammonia N	24.6–26.8	13.1–16.5
Arginine N	4.6– 5.7	8.2–12.9
Total basic N	11.0–14.4	18.0–26.2
Amino N of filtrate	51.4–54.1	53.4–58.2
Non-amino N of filtrate	4.4–10.7	2.6– 9.5

The range in gliadin analyses is fairly small and one might almost be justified in attributing the differences to experimental errors. The very wide variations noted in the data for glutenin, however, can scarcely be attributed to this cause. They might be due to a combination of differences of technique of preparation and analysis. In order to get information on the error to be expected in two samples obtained by one method, two preparations of gliadin and glutenin were made by the Blish and Sandstedt (3) method. These were analyzed in duplicate and the results are shown in Table II. It is apparent that the variation between preparations is no greater than the variation in duplicate analyses. On the basis of these data it may be concluded tentatively that it is possible to replicate preparations of proteins in one laboratory. The question whether different men using the same procedure in different laboratories could make as good replication remains to be investigated.

At this point it was planned to prepare and analyze proteins from a series of flours of graded strength in order to ascertain if any chemical differences could be discovered. However, in deciding to prepare flour proteins one is at once confronted with choice of method, as there are a number of procedures,

TABLE II

VAN SLYKE NITROGEN DISTRIBUTION OBTAINED WITH DUPLICATE PREPARATIONS BY THE BLISH AND SANDSTEDT (1929) METHOD (USING 0.007 *N* ACETIC ACID)

Nitrogen fraction	Preparation A			Preparation B		
	1	2	Mean	1	2	Mean
Gliadin						
Ammonia N	25.80	25.85	25.82	25.83	25.62	25.72
Total humin N	0.85	0.68	0.77	0.87	0.82	0.84
Total basic N	10.72	10.33	10.53	11.22	11.07	11.14
Arginine	5.41	5.39	5.40	5.17	5.30	5.23
Cystine	0.61	0.96	0.79	0.63	0.67	0.65
Histidine	4.31	3.30	3.79	4.67	4.45	4.56
Lysine	0.39	0.70	0.55	0.75	0.15	0.70
Total filtrate N	62.98	62.84	62.81	61.91	62.05	61.98
Amino	53.59	53.48	53.53	53.86	53.98	53.92
Non-amino	9.39	9.16	9.28	8.05	8.07	8.06
Total	100.35	99.52	99.93	99.83	99.56	99.68
Glutenin						
Ammonia N	21.46	21.31	21.38	20.58	20.58	20.58
Total humin N	1.36	1.44	1.40	1.59	1.40	1.49
Total basic N	17.87	18.45	18.16	17.77	17.87	17.82
Arginine	8.47	8.39	8.43	8.52	8.69	8.60
Cystine	0.96	0.92	0.94	0.89	0.96	0.93
Histidine	1.52	1.70	1.61	1.49	1.58	1.54
Lysine	6.92	7.44	7.18	6.87	6.64	6.75
Total filtrate N	60.14	59.22	59.68	60.63	60.53	60.58
Amino	54.15	54.26	54.20	54.63	53.91	54.27
Non-amino	5.99	4.96	5.48	6.00	6.62	6.31
Total	100.83	100.47	100.60	100.87	100.36	100.47

each of which has merits. Being unable to decide the relative value of the various methods of preparation a comparison of them was undertaken in the hope that the results would give a basis on which to make a choice. Accordingly a large uniform sample of commercially milled first patent unbleached flour was obtained and, from this, gliadin and glutenin were prepared by the following methods.

I. Osborne Method

In this preparation the method used was that outlined by Osborne (20). This is the oldest and most widely used procedure and therefore has the advantage that the results obtained can be compared with those of other investigators. In this and subsequently discussed preparations 1000 gm. of flour was mixed to a stiff dough with 600 cc. of tap water and allowed to stand under water for two hours at the end of which time the crude gluten was washed out under a slow stream of water from the tap. The gluten was then left in tap water for 16 hr. and finally ground in a meat chopper. In all preparations, the finely ground gluten was placed in a four litre beaker with three litres of the dispersion medium and stirred continuously by means of motor driven glass stirring rods for approximately five hours, allowed to settle

overnight, the supernatant liquid decanted, more of the dispersion medium added, and the process repeated until extraction or dispersion was complete. All solutions were clarified by use of the Sharples supercentrifuge at a speed of 38000-40000 r.p.m. Alcoholic solutions were concentrated *in vacuo* at a temperature below 35° C.

In Preparation I the first extraction was made with three litres of 70% ethyl alcohol, followed by four extractions each with two litres of alcohol. The fourth extract contained 0.014% nitrogen and was discarded. Thereafter five more extractions were made, each with one-litre portions of the 70% alcohol, in order to remove the last traces of gliadin. The tenth and final extract showed only a trace of nitrogen. The alcoholic solution was centrifuged and concentrated to a thick syrup which was poured into 10 litres of distilled water containing five grams of NaCl. The precipitate was redissolved in one litre of 70% alcohol and this solution was again concentrated to a syrup from which gliadin was precipitated as before. The third precipitation was made by pouring the syrup into 400 cc. of a mixture of two parts absolute alcohol and one part anhydrous ether. Thereafter the protein was extracted twice with the absolute alcohol-ether mixture, three times with anhydrous ether, and finally dried

TABLE III

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD I (OSBORNE'S METHOD)

Analysis

	Gliadin	Glutenin
Amount recovered, gm.	28	10
Moisture, %	3.25	2.50
Ash, %	0.17	0.50
Nitrogen, %	16.36	15.83
Nitrogen (corr. for moist. and ash), %	16.93	16.84

Nitrogen distribution as determined by the Van Slyke method, %

Nitrogen fraction	Gliadin			Glutenin		
	1	2	Mean	1	2	Mean
Ammonia N	25.45	25.50	25.48	12.51	12.90	12.70
Total humin N	0.88	0.81	0.84	2.01	2.06	2.04
Acid insoluble	0.23	0.23	0.23	0.74	0.83	0.78
Acid soluble	0.24	0.22	0.23	0.84	0.74	0.79
Phosphotungstic	0.41	0.36	0.38	0.43	0.49	0.47
Total basic N	10.69	10.76	10.72	23.62	23.97	23.79
Arginine	5.23	5.22	5.22	12.81	12.50	12.65
Cystine	0.48	0.50	0.49	0.44	0.45	0.44
Histidine	4.32	4.35	4.33	6.89	7.33	7.11
Lysine	0.66	0.69	0.68	3.48	3.69	3.59
Total filtrate N	62.22	62.28	62.25	61.03	60.88	60.95
Amino	52.53	52.10	52.32	51.74	52.90	52.32
Non-amino	9.69	10.18	9.93	9.29	7.98	8.63
Total	99.24	99.32	99.29	99.17	99.81	99.49

in vacuo at 35° C. for 12-15 hr. It was then ground in a ball mill, re-dried *in vacuo* as a powder and after removal was carefully stoppered.

Glutenin was prepared by dissolving the residue from the gliadin extraction in 0.025 *N* NaOH. This solution was supercentrifuged and treated with dilute hydrochloric acid until maximum flocculation occurred. The precipitate was collected on a linen filter cloth and extracted with one litre of 70% alcohol after which it was redispersed in 0.025 *N* NaOH, centrifuged and precipitated. Two reprecipitations were made. The final precipitate was washed once with 95% alcohol, twice with absolute alcohol-ether mixture, and three times with anhydrous ether. It was then dried *in vacuo* at 35° C. for 12-15 hr., ground, re-dried, and stoppered.

Van Slyke analyses in duplicate were made on these preparations, according to the usual procedure. The data are given in Table III.

II. Osborne Method Without Dispersal of the Glutenin in any Solvent

In this preparation the gliadin was isolated as in the previous method. It was proposed to make a combination of the Osborne method and the Blish and Sandstedt acetic acid method, by extracting the gliadin with 70% alcohol and dispersing the residue in acetic acid in place of sodium hydroxide solution.

TABLE IV

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD II (NO DISPERSION OF THE GLUTENIN)

Analysis	Gliadin	Glutenin
Amount recovered, gm.	40	33
Moisture, %	3.35	2.84
Ash, %	0.06	0.25
Nitrogen, %	16.39	11.86
Nitrogen (corr. for moist. and ash), %	16.97	12.23

Nitrogen distribution as determined by the Van Slyke method, %

Nitrogen fraction	Gliadin			Glutenin		
	1	2	Mean	1	2	Mean
Ammonia N	25.66	25.58	25.62	18.42	18.39	18.40
Total humin N	0.82	0.76	0.79	2.62	2.46	2.54
Acid insoluble	0.22	0.24	0.23	1.53	1.71	1.62
Acid soluble	0.25	0.12	0.18	0.69	0.45	0.57
Phosphotungstic	0.35	0.40	0.38	0.40	0.30	0.35
Total basic N	10.74	10.27	10.50	18.59	17.62	18.10
Arginine	5.44	5.57	5.50	8.45	8.74	8.59
Cystine	0.62	0.73	0.67	0.72	0.79	0.76
Histidine	4.05	3.34	3.70	4.84	3.79	4.31
Lysine	0.63	0.63	0.63	4.58	4.30	4.44
Total filtrate N	61.81	62.64	62.23	61.00	61.48	61.24
Amino	52.51	52.54	52.53	53.43	54.03	53.73
Non-amino	9.30	10.10	9.70	7.57	7.45	7.51
Total	99.03	99.25	99.14	100.63	99.95	100.28

It was found, however, that when 0.07 *N* acetic acid was added no apparent dispersion took place. The residue absorbed the solution and swelled until it completely filled the four-litre container with a jelly-like mass. This was transferred to a 14-litre vessel and seven litres more acetic acid solution was added. After continuous stirring for five hours, the solution showed 0.011% nitrogen or approximately six grams of protein dissolved in the 10 litres. Various concentrations of the acid solution were tried with small portions of the material but dispersion could not be effected. The mass was drained on a linen filter and then washed three times with eight-litre portions of distilled water with very vigorous stirring in an effort to separate by mechanical means as much starch as possible. As the residue was still exceedingly bulky *N*/14 NaOH solution was carefully added until the solution remained just faintly acid, the volume of the residue being thus reduced from about four litres to 300 cc. This was then reground in the meat chopper and washed with continuous stirring in three successive portions of 10 litres of distilled water. It was finally drained and dried in the usual manner.

Analyses of these two proteins are given in Table IV. The glutenin was very low in nitrogen, the value being 12.23%, which indicates that it probably contained a high percentage of starch, probably about 28%. No work has been done to determine the effect on the nitrogen distribution of this concentration of starch. Hart and Sure (7) carried out the Van Slyke analysis on a mixture of 2.4 gm. casein and 12 gm. starch but this represents a very much greater degree of contamination than in the sample in question here. On consideration of the available data regarding the effect of carbohydrates on the nitrogen distribution Larmour (15) concluded that the humin nitrogen and filtrate nitrogen are most affected and that the basic fractions may be regarded as fairly reliable.

In the glutenin of Method II the humin nitrogen shows an average value of 2.54% which, compared to the value 2.04% for glutenin by Osborne's method, would indicate that the impurity was not having a great effect on the distribution, as the humin nitrogen fraction is the one that is most profoundly affected

TABLE V

COMPARISON OF DATA OBTAINED WITH DUPLICATE PREPARATIONS OF GLIADIN MADE BY THE OSBORNE METHOD

Nitrogen fraction	Preparation I			Preparation II			Diff. of means I-II
	1	2	Mean	1	2	Mean	
Ammonia N	25.45	25.50	25.48	25.66	25.58	25.62	−0.14
Total humin N	0.88	0.81	0.84	0.82	0.76	0.79	0.05
Total basic N	10.69	10.76	10.72	10.74	10.27	10.50	0.22
Arginine	5.23	5.22	5.22	5.44	5.57	5.50	−0.28
Cystine	0.48	0.50	0.49	0.62	0.73	0.67	−0.18
Histidine	4.32	4.35	4.33	4.05	3.34	3.70	0.63
Lysine	0.66	0.69	0.68	0.63	0.63	0.63	0.05
Total filtrate N	62.22	62.28	62.25	61.81	62.61	62.23	0.02
Amino	52.53	52.10	52.32	52.51	52.54	52.53	−0.21
Non-amino	9.69	10.19	9.93	9.30	10.10	9.70	0.23
Total	99.24	99.32	99.29	99.03	99.25	99.14	

by carbohydrates. It is quite probable that this relatively low percentage of carbohydrate did not materially alter the nitrogen distribution particularly with respect to the basic fractions.

The gliadin preparations in Methods I and II were prepared by the same technique, therefore the analytical data for the two samples may be used to examine the agreement of duplicate preparations of this protein by the Osborne method. For convenience in making comparison the data are reproduced in Table V. Inspection of these data shows that the differences between all four analyses are no greater than would be found in four replicate analyses of one individual preparation. It is evident, therefore, that only very small errors may be expected in replicate preparations of gliadin by the Osborne method.

III. Method of Blish and Sandstedt (2)

This method differs from Osborne's in that the crude gluten is initially all dispersed in dilute sodium hydroxide solution. The solution is then treated with 95% alcohol until the alcoholic strength reaches 70% and from this the glutenin is precipitated by adding acid until the isoelectric pH of glutenin is reached. The gliadin is recovered from the filtrate in the usual manner. This method has the advantage that it is a more rapid one and does not involve long exposure to alcohol.

TABLE VI

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD III, (ALL GLUTEN DISPERSED IN ALKALI)

Analysis	Gliadin	Glutenin
Amount recovered, gm.	21	13
Moisture, %	1.62	2.18
Ash, %	0.25	0.41
Nitrogen, %	16.50	16.07
Nitrogen (corr. for moist. and ash), %	16.82	16.50

Nitrogen distribution as determined by the Van Slyke method, %

Nitrogen fraction	Gliadin			Glutenin		
	1	2	Mean	1	2	Mean
Ammonia N	24.99	25.18	25.05	15.26	14.85	15.06
Total humin N	0.82	1.03	0.92	1.60	1.68	1.64
Acid insoluble	0.30	0.44	0.37	0.66	0.73	0.69
Acid soluble	0.29	0.41	0.35	0.59	0.64	0.62
Phosphotungstic	0.23	0.18	0.21	0.35	0.31	0.33
Total basic N	14.39	13.95	14.17	21.02	20.36	20.69
Arginine	6.01	6.29	6.15	11.91	11.60	11.75
Cystine	0.62	0.67	0.64	0.68	0.72	0.70
Histidine	6.17	5.36	5.77	3.87	3.32	3.60
Lysine	1.59	1.63	1.61	4.56	4.72	4.64
Total filtrate N	59.86	60.41	60.13	62.47	62.49	62.48
Amino	55.31	53.54	54.42	54.63	53.91	54.27
Non-amino	4.55	6.87	5.71	7.84	8.58	8.21
Total	100.06	100.57	100.27	100.35	99.38	99.86

Three litres of 0.025 *N* NaOH was used in the initial treatment. Most of the gluten dispersed leaving only a small residue which was extracted with two successive one-litre portions of sodium hydroxide solution. The third extract contained 0.015 gm. nitrogen per 100 cc. These last two solutions were concentrated *in vacuo* at 35° C. to a volume of 55 cc. and added to the first extract. The total solution, or more properly, dispersion, was centrifuged at 39000 r.p.m. at the slowest rate of feeding, and then made up to a concentration of 70% by addition of the necessary amount of 95% ethyl alcohol. Hydrochloric acid was added until the point of maximum flocculation was reached. The filtrate was centrifuged before concentration in order to remove any glutenin that had not settled out or that had escaped the linen filter cloth. Purification and drying of the proteins was carried out in the manner described in Method I. Analytical data are given in Table VI.

IV. Blish and Sandstedt (3) Method with 0.007 *N* Acetic Acid

There is much evidence to show that changes in the chemical properties of proteins occur when they are exposed to alkali. Knaggs and Schryver (13, 14) and later Knaggs (12) reported the observation that the diamino nitrogen fraction of gelatin varied according to the treatment given the collagen from which it was prepared. After a 60-day exposure to alkali, treatment with 0.5% HCl for one day prior to preparation of the gelatin produced a significant lowering of the diamino fraction. Blish and Sandstedt (3) showed that the basic nitrogen fraction of glutenin inversed with increasing strength of the alkali solution. This increase was accompanied by a decrease of ammonia nitrogen. On account of this variability in chemical composition of the glutenin prepared in alkaline medium they recommend a method of preparation in which dilute acetic acid is used as the dispersion medium in place of sodium hydroxide solution.

They state that difficulty was encountered in attempting to effect acid dispersion of gluten but that this was accomplished in very dilute acetic acid. Unfortunately they did not specify the strength of acid used. Rather empirically a concentration of 0.007*N* acetic acid was chosen and the ground wet gluten was treated with three litres. After five hours stirring there resulted a rather viscous milky dispersion which, on standing overnight, was little clearer. There was, however, a $\frac{1}{4}$-in. layer of residue left on the bottom of the vessel. The supernatant liquid was decanted and centrifuged. At the lower end of the centrifuge bowl there was found the usual deposit of fairly dry material characteristic of starch removal, but at the upper end there was quite a large amount of impure protein. This was separated and returned to the extraction vessel and treated with two litres of the acid. This extracted only a small fraction of the residue which, after a third treatment, was discarded although it was shown to contain some protein.

Blish and Sandstedt (3) remarked that a satisfactory separation of glutenin could not be made when ethyl alcohol was used in place of methyl alcohol. Two 100-cc. portions of the acetic acid-protein solution were made up to 70% alcohol by addition of ethyl and methyl alcohol respectively. Upon almost

complete neutralization by means of $N/14$ NaOH no differences in the rate of precipitation or in the nature of the precipitate obtained could be observed; therefore ethyl alcohol was used subsequently. This precipitation of glutenin is more difficult than in the other methods, due perhaps to the fact that the coagulum forms somewhat more slowly and there is a tendency to go past the optimum pH. However, at the correct pH there appears to be a very complete separation leaving practically a water-clear filtrate. The glutenin so obtained showed characteristics similar to those observed by Blish and Sandstedt (3), *i.e.*, it was more coherent and rubbery than other preparations and resembled the original gluten a great deal, although the tendency to coalesce was less marked than in gluten. In proceeding to purify this initial precipitate it was found that it would not dissolve in 0.007 N acetic acid to any appreciable extent. It was, therefore, ground in the chopper and extracted several times with 70% ethyl alcohol and finally dehydrated and ground in the usual manner. The gliadin was prepared from the filtrate in the usual manner and presented no exceptional difficulties.

TABLE VII

ASH, MOISTURE, AND NITROGEN OF REPLICATE PREPARATIONS BY METHOD IV, BLISH AND SANDSTEDT'S (1929) ACETIC ACID DISPERSION (USING 0.007 N ACETIC ACID)

	Gliadin		Glutenin	
	Prep. A	Prep. B	Prep. A	Prep. B
Amount obtained, gm.	32	31	16	12
Moisture, %	3.58	3.69	6.17	5.94
Ash, %	0.19	0.36	0.43	0.97
Nitrogen, %	16.33	16.22	16.08	15.97
Nitrogen, % (corrected for ash and moisture)	16.96	16.91	17.20	17.15

Replicate preparations were made by this method because it was thought that failure to dissolve all of the crude gluten might introduce appreciable errors in composition of the glutenin. The data for the Van Slyke analyses of the replicates are given in Table III and have been discussed from the standpoint of error involved in replication. The results appear to be quite reproducible by the method used here. Data for ash, moisture, and nitrogen which were omitted from Table III are shown in Table VII. These two samples of glutenin were higher in nitrogen than any of the other preparations made.

V. Blish and Sandstedt (3) Method, Using Acetic Acid 0.07 *N* in Place of 0.007 *N*

In this preparation 0.07 N acetic acid was used to disperse the wet crude gluten. A greater fraction of it actually dispersed but even after five successive treatments there was left a small residue from which protein could be extracted with 0.025 N NaOH. The quantity obtained by this latter treatment was much too small for analysis.

The glutenin precipitated from the acetic acid-70% alcohol solution was much less coherent and elastic than that obtained with use of 0.007 N acetic acid, and resembled more nearly the preparations from alkaline dispersions.

About 75% of this glutenin could be redispersed in 0.07*N* acetic acid but the remainder proved particularly resistant to acid dispersion. The reprecipitation of the glutenin was very difficult and only a small fraction was finally obtained. Without further attempts at dispersion the material was treated several times with 70% alcohol and finally dried in the usual way.

The gliadin gave some difficulty because in the initial precipitation it failed to coagulate and remained as a fine milky suspension. It was found necessary to neutralize most of the acetic acid left, and to increase the amount of sodium chloride to 20 gm. per 10 litres. Under these conditions a considerable amount was precipitated. Part of the gliadin still suspended was recovered from the upper part of the centrifuge bowl after the filtrate had been slowly fed through

TABLE VIII

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD V (DISPERSION IN 0.07 *N* ACETIC ACID)

Analysis	Gliadin	Glutenin
Amount recovered, gm.	18	4
Moisture, %	1.48	4.14
Ash, %	0.31	0.59
Nitrogen, %	16.35	15.29
Nitrogen (corr. for moist. and ash), %	16.65	16.05

Nitrogen distribution as determined by the Van Slyke method, %				
Nitrogen fraction	Gliadin			Glutenin*
	1	2	Mean	
Ammonia N	25.40	24.86	25.13	16.07
Total humin N	1.03	1.01	1.02	2.14
Acid insoluble	0.47	0.47	0.47	0.77
Acid soluble	0.35	0.33	0.34	1.12
Phosphotungstic	0.21	0.21	0.21	0.25
Total basic N	13.84	14.47	14.15	20.50
Arginine	6.33	6.11	6.22	12.12
Cystine	1.05	1.03	1.04	0.63
Histidine	5.22	6.12	5.67	3.14
Lysine	1.24	1.21	1.22	4.61
Total filtrate N	59.34	60.35	59.85	61.48
Amino	52.65	52.57	52.61	55.30
Non-amino	6.69	7.78	7.24	6.18
Total	99.61	100.69	100.15	100.19

**As but 4 gm. of this preparation was obtained the values reported are for a single determination.*

the machine at 40000 r.p.m. From a Kjeldhal determination made on the filtrate it was estimated that about 20 gm. of gliadin was lost at this point. The data, in Table VIII, show that the gliadin was lower in nitrogen than any of the other preparations of this protein and, excepting Preparation II, this holds for the glutenin also.

Discussion of the Results

In order to facilitate comparison of all the results obtained, the mean values of analyses on the various preparations have been brought together in Table IX. One is impressed immediately with the uniformity of results obtained with the gliadin. Were it not for the variations in the total basic and non-amino filtrate fraction, the analyses exhibit no greater variation than might be expected from replicate analyses of one preparation. The range in nitrogen

TABLE IX

SUMMARY OF ANALYSES MADE ON THE VARIOUS PREPARATIONS

Method of preparation	N in protein	Ammonia N	Humin N	Total basic N	Arginine N	Cystine N	Histidine N	Lysine N	Filtrate N	
									Amino	Non-amino
Gliadin										
I. Osborne's method	16.9	25.5	0.8	10.7	5.2	0.5	4.3	0.7	52.3	9.9
II. Osborne's method	17.0	25.6	0.8	10.5	5.5	0.7	3.7	0.6	52.5	9.7
III. Blish and Sandstedt (1924)	16.8	25.0	0.9	14.2	6,2	0.6	5.8	1.6	54.4	5.7
IV. Blish and Sandstedt (1929) acetic acid 0.007 *N* Prep. 1	17.0	25.8	0.8	10.5	5.4	0.8	3.8	0.6	53.5	9.3
Prep. 2	16.9	25.7	0.8	11.1	5.2	0.6	4.6	0.7	53.9	8.1
V. Blish and Sandstedt (1929) acetic acid 0.07 *N*	16.6	25.1	1.0	14.2	6.2	1.0	5.7	1.2	52.6	7.2
Glutenin										
I. Osborne's method	16.8	12.7	2.0	23.8	12.6	0.4	7.1	3.6	52.3	8.6
II. Osborne's method	12.2	18.4	2.5	18.1	8.6	0.8	4.3	4.4	53.7	7.5
III. Blish and Sandstedt (1924)	16.5	15.1	1.6	20.7	11.8	0.7	3.6	4.6	54.3	8.2
IV. Blish and Sandstedt (1929) acetic acid 0.007 *N* Prep. 1	17.2	21.4	1.4	18.2	8.4	0.9	1.6	7.2	54.2	5.5
Prep. 2	17.2	20.6	1.5	17.8	8.6	0.9	1.5	6.8	54.3	6.3
V. Blish and Sandstedt (1929) acetic acid 0.07 *N*	16.1	16.1	2.1	20.5	12.1	0.6	3.1	4.6	55.3	6.2

was 16.6-17.0%, the low value being for gliadin by Method V (0.07 *N* acetic acid). The other five preparations showed a variation of 0.2%. The range for ammonia nitrogen was 25.0% to 25.8% which was within the limits of experimental error. The total humin nitrogen was quite constant, indicating that carbohydrate contamination was of approximately the same extent in all cases. The total basic nitrogen in Preparations III (gluten initially dispersed in alkali) and V (0.07 *N* acetic acid) was approximately 40% higher than in the others. This was made up of increases in arginine, histidine, and lysine nitrogen. These two preparations also had the lowest ammonia-nitrogen fraction and the lowest non-amino filtrate nitrogen. Furthermore, these two analyses checked as closely as duplicate determinations on the same sample. It appears that prolonged exposure to 0.025 *N* NaOH or to 0.07 *N* acetic acid produces practically the same sort of modification of the nitrogen distribution. Haugaard and Johnston (8) have shown that gliadin can be fractionated on the basis of solubility and that the most readily soluble fraction is highest in non-amino nitrogen and lowest in ammonia nitrogen. As the

preparations under discussion are lowest in both ammonia nitrogen and non-amino nitrogen, the differences observed cannot be attributed to fractionation on the basis of solubility in the different media employed.

Considering the glutenins, it is evident that the preparations made by Method IV, using 0.007 *N* acetic acid, were the purest. They were highest in nitrogen and lowest in humin nitrogen. The only two preparations that came at all close to checking in respect to ammonia and basic nitrogen are III (alkali dispersion of the gluten) and V (using 0.07 *N* acetic acid). Except for non-amino filtrate nitrogen and, to slighter extent, ammonia nitrogen, these might be taken for replicates. This tends to confirm the conclusion reached in regard to gliadin, namely, that long exposure to alkali and exposure to acid as strong as 0.07 *N* acetic acid tend to produce the same sort of variation.

There is a great range of variation in ammonia nitrogen, total basic nitrogen, and arginine nitrogen in these preparations. The Osborne method gave low ammonia nitrogen and high basic nitrogen, while Method IV (0.007 *N* acetic acid) gave high ammonia nitrogen and low basic nitrogen. It is interesting to note that the relation of ammonia nitrogen and basic nitrogen in these preparations, with the exception of Preparation IV, were virtually interdependent so that one varied inversely as the other. This is brought out clearly in Table X. Thus in four of the five preparations the sum of these two fractions was almost

TABLE X

THE RELATIONSHIP OF AMMONIA NITROGEN AND TOTAL BASIC NITROGEN IN THE GLUTENIN PREPARATIONS

Preparation	Ammonia N	Total Basic N	Sum
I	12.7	23.8	36.5
II	18.4	18.1	36.5
III	15.1	20.7	35.8
IV (mean)	21.0	18.0	39.0
V	16.1	20.5	36.6

TABLE XI

VALUES FOR AMMONIA NITROGEN AND BASIC NITROGEN IN VARIOUS GLUTENIN PREPARATIONS

Observer	Source of flour	Method	Ammonia N	Basic N	Sum
Blish (1)	Spring wheat	Alkali	16.5	18.0	34.5
Blish	Soft wheat	Alkali	16.2	18.9	35.1
Cross and Swain (5)	Idaho wheat	Alkali	15.6	22.6	38.2
Cross and Swain	Patent	Alkali	16.0	23.0	39.0
Cross and Swain	Club	Alkali	14.2	26.0	40.2
Cross and Swain	Forty-fold	Alkali	13.1	26.2	39.3
Hoffman and Gortner (10)	Patent	Alkali	13.6	21.9	35.5
Larmour (15)	Patent	Alkali	14.8	18.8	33.6
Csonka and Jones (6)	(α—glutelin)	Alkali	17.8	21.3	39.1
Csonka and Jones	(β—glutelin)	Alkali	11.1	24.6	35.7
Cook and Alsberg (4)	Spring wheat	Alkali	17.9	18.2*	36.1
Cook and Alsberg	Spring wheat	Urea	16.4	18.7*	35.1
Cook and Alsberg	Spring wheat	Urea (4 preps.)	19.5	14.5*	34.0
Authors Prep. I	Spring wheat	Alkali	12.7	23.8	36.5
Authors Prep. II	Spring wheat	No dispersion	18.4	18.1	36.5
Authors Prep. III	Spring wheat	Alkali	15.1	20.7	35.8
Authors Prep. IV	Spring wheat	Acetic acid (0.007 *N*)	21.0	18.0	39.0
Authors Prep. V	Spring wheat	Acetic acid (0.07 *N*)	16.1	20.5	36.6

**Without the correction for arginine in the filtrate from the bases.*

constant. This would indicate that if by any manipulation the ammonia nitrogen were decreased, the basic nitrogen would be increased by the same amount. This is admirably shown in Blish and Sandstedt's (3) data on the influence of alkali of different strengths. Their Tables III and IV show that the decrease in amide nitrogen is almost exactly equal to the corresponding increase in basic nitrogen. It should be noted that no change occurs in filtrate nitrogen comparable to the changes in ammonia and basic nitrogen. That this is not a fortuitous relationship is indicated by the data of a number of investigators given in Table XI. The correlation coefficient $r = -0.84$ indicates a very marked tendency for the basic nitrogen to vary inversely as the ammonia nitrogen.

TABLE XII

THE RELATIONSHIP OF AMMONIA NITROGEN AND TOTAL BASIC NITROGEN IN THE GLIADIN PREPARATIONS

Preparation	Ammonia N	Basic N	Sum
I	25.5	10.7	36.2
II	25.6	10.5	36.1
III	25.0	14.2	39.2
IV (mean)	25.8	10.8	36.6
V	25.1	14.2	39.3
			Mean 37.5%

Furthermore, attention should be directed to the fact, shown in Table XII, that the sum of these two fractions in gliadin tends to approach the same value as in glutenin. The mean value of the sum for the glutenins in Table XI is 37.0% and for the gliadins in Table XII it is 37.5%. From all analyses available on wheat gliadin we obtain 38.0% as the mean value of the sum of ammonia- and basic nitrogen. It seems probable, therefore, that this sum may be a more important constant than either of its components.

The explanation of this relation between these two fractions that probably first comes to mind is that, in the course of treatment of the protein with alkali, ammonia is lost. If this occurred it would lower the nitrogen content of the protein and increase the percentage of all but the ammonia fraction. It will be observed that in this work Preparation IV, having the highest nitrogen in the protein, had the highest ammonia nitrogen fraction, but no definite conclusion on this basis can be reached as we have no means for accurately estimating the carbohydrate impurities present in the various preparations. Moreover, it can be shown by calculation that the theoretical change wrought in the distribution of the fractions does not correspond to the experimental facts. Taking, as a case, the glutenin preparation of highest ammonia nitrogen, namely, Preparation I of Method IV, and assuming that it represents the real protein, suppose that in course of isolation it lost 54.7% of its ammonia nitrogen. The modified protein would then, upon analysis, give the nitrogen distribution shown in column 2 of Table XIII. Thus if one attempts to explain the difference between the glutenins of Method IV and Method I, by assuming a loss of 54.7% of the ammonia nitrogen due to action of the alkaline solution, the theoretical distribution calculated on the basis of such a loss does not agree with the results as actually found with a sample of glutenin

having 12.7% ammonia nitrogen. In place of 19.8% basic nitrogen calculated there was found 23.8%, and in place of 66 0% filtrate nitrogen calculated, 60.9%.

As will be shown later there is loss of nitrogen as ammonia when glutenin is treated with alkali, but this alone cannot account for the increase in basic nitrogen. Leaving aside glutenin IV, the others all show increase of basic nitrogen commensurate with increase of ammonia nitrogen and relatively slight variation in filtrate nitrogen. Loss of nitrogen in course of isolation of the protein will not therefore explain the differences in nitrogen distribution noted in Preparations I, II, III, and V.

TABLE XIII

CALCULATIONS SHOWING THE EFFECT ON THE NITROGEN DISTRIBUTION OF GLUTENIN (AVERAGE, METHOD IV) OF A LOSS OF 54.7% OF ITS AMMONIA NITROGEN

Original		After loss of 46.4% of its ammonia nitrogen, %	Nitrogen distribution of glutenin by Osborne's method, %
Ammonia N	21.0	12.7	12.7
Humin N	1.4	1.5	2.0
Basic N	18.0	19.8	23.8
Filtrate N	60.1	66.0	60.9

On the other hand, if it is true that the ammonia nitrogen and basic nitrogen fractions are inversely proportional, a new interpretation of the ammonia nitrogen must be found. If this fraction is from amide groupings only, it is difficult to see why a treatment resulting in lowered ammonia nitrogen should produce a corresponding increase in the basic fraction. Without more data it is rather useless to speculate concerning the reason for this remarkable relationship. It seems fairly evident, however, that much weight should not be attached to either ammonia nitrogen or total basic nitrogen of preparations made by methods involving use of alkali or of 0.07 *N* acetic acid.

TABLE XIV

RESULTS OBTAINED BY TREATING GLUTENIN OF METHOD IV (BLISH AND SANDSTEDT'S (1929) ACETIC ACID METHOD) FIRST WITH 0.1% SODIUM HYDROXIDE FOR ONE WEEK AND SUBSEQUENTLY HYDROLYZING AND FRACTIONATING THE RESIDUE

Fraction	Original values of glutenin of Method IV (Prep. A)	Values obtained on treated sample as % of initial N	Values calculated on basis of 4.8% N lost in initial treatment
N removed by treatment with 0.1% NaOH		4.80	
Ammonia N after acid hydrolysis	21.38	16.63	17.47
Total ammonia N		21.43	
Humin N	1.40	1.41	1.48
Total basic N	18.16	17.99	18.90
Arginine N	8.43	8.05	8.46
Sum of ammonia N and basic N	39.54		36.37

In order to ascertain if ammonia is lost when a protein is exposed to alkali, 3 gm. of glutenin of Preparation IV (0.007 *N* acetic acid) was dissolved in 250 cc. of 0.025 *N* NaOH in a Claissen flask which was connected up as for the ammonia nitrogen determination. This was left, protected by toluene for

one week, at the end of which time it was distilled *in vacuo* at 40° C. It was found that 4.81% nitrogen was removed by this treatment. The residue was neutralized, the solution evaporated and the protein hydrolyzed in the usual manner. Results of the analysis are shown in Table XIV. In the third column of Table XIV are shown the values calculated to the basis of the nitrogen content of the protein after it had been submitted to cold digestion with 0.025 *N* NaOH for seven days. There was a marked decrease in ammonia nitrogen and a slight increase in basic nitrogen. By reference to column 2 it is evident that with respect to the initial protein these values are all spurious, as the value of the ammonia fraction appears to be increased, whereas in reality there was virtually no change in it attributable to the treatment by 0.025 *N* NaOH. It should be noticed too that the sum of ammonia- and basic-nitrogen has been reduced from 39.54 to 36.37%, the latter value being comparable to that obtained for the other four preparations in the series (see Table X).

Comparing the first and third columns of Table XIV it can be seen that the reduction in the ammonia-nitrogen fraction is not accompanied by a corresponding increase in the basic nitrogen fraction as was observed by Blish and Sandstedt (3) in their experiments with alkali of different strengths. In this experiment it appears that the only effect of the digestion in alkali was a loss of nitrogen from the protein. There was no significant change in the basic fraction. The initial loss of nitrogen, however, caused a redistribution of percentages for the remaining nitrogen, resulting in values that were spurious in respect to the original protein. Unfortunately only one concentration of alkali was used and we have no information concerning the effect of different strengths of sodium hydroxide on the distribution in column 3.

The question of choice of the five methods used seems to be quite definitely settled by this experiment. The protein prepared by Method IV, having a high initial ammonia-nitrogen fraction,loses nitrogen when brought into contact with alkali of the strength ordinarily used. This is doubtless a loss of ammonia nitrogen because the basic fraction does not seem to be affected. The loss of a little ammonia nitrogen from a protein would probably not be a serious matter by itself, especially as we do not know the origin of this fraction, but the resulting redistribution of all the other fractions constitutes an important source of error particularly as there is some evidence for believing that the amount of nitrogen lost from a protein is proportional to the time of exposure to the alkaline solution. The time during which a protein is kept in solution is most difficult to control because it depends so much upon the operator's success in making filtrations, or upon the operation of the centrifuge, or upon the speed with which the isoelectric point can be reached in making the precipitation. The use of alkaline solutions in preparing glutenin thus introduces possibilities of wide errors in the final analyses and, therefore, we endorse Blish and Sandstedt's (3) conclusion that it should be strictly avoided. On the other hand use of 0.07 *N* acetic acid gives results similar to those obtained in preparations

involving alkali. Of the five methods applied in this study Method IV, involving use of 0.007 *N* acetic acid, seems to be the best.*

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**Recently Cook and Alsberg (4) have published an account of results obtained with glutenin prepared in neutral 30% urea solutions. Using the sulphydryl test as a criterion they concluded that less denaturation occurs by use of urea solutions than when alkali or acid are used. The ammonia nitrogen values in their preparations are somewhat lower than in our preparation by Method IV using 0.007 N acetic acid. This, however, cannot be used to compare the methods as the proteins were prepared from different samples of flour.*

THE UTILITY OF COOKED POTATO IN BAKING BREAD AND ITS RELATION TO CRUDE PROTEIN AND BAKING STRENGTH[1]

By R. H. Harris[2]

Abstract

Two commercially milled flours, baked by a formula including the liquid drained from boiled sliced potato, gave loaves showing a progressive increase in color and loaf volume with increasing quantities of the liquid. Dried mashed potato, containing all the original material, also caused an increase in volume with each increment of potato.

A number of doughs, including controls and doughs treated with varying proportions of mashed potato, showed increased gas production with increasing quantities of potato. The gas lost from the doughs also tended to increase in the same manner but was less than the increase in the total gas evolved. The volume of the doughs accordingly increased with increasing potato concentration.

A series of 10 commercial flours of various types was baked by the simple basic formula and by one including 5, 10 and 40% of cooked mashed potato in addition. A baking was also made of a blend of 50 gm. of flour to 50 gm. of potato and another using 1% diastatic malt and 0.001% $KBrO_3$ in addition to the simple ingredients. The resultant loaf volumes were found to increase as more potato was added. The higher protein flours gave larger loaves throughout. The color of the loaves decreased with the higher potato concentrations, the grain and texture of these loaves also being very poor. Loaf volumes and baking score, calculated on a basis of 100 gm. total material, decreased above 30% potato concentration. Loaves baked with more than 10% potato to 100 gm. flour were of inferior grain and texture. Crude protein and loaf volume were significantly related throughout.

Introduction

Cooked potato is extensively used in bread making by many people who are accustomed to produce all or part of their bread requirements. The liquid drained from the potato, "potato water," is frequently used and added to the yeast before mixing the dough or to the flour in place of ordinary water. In other cases, the mashed potato is mixed in with the flour and other ingredients, or a mixture of potato water and potato is used with either sponge or straight-dough methods with apparently beneficial results. This widespread use of cooked potato as a flour improver would seem to indicate some inherent virtue in this vegetable with respect to its influence on bread making in general.

Jago (7) mentions the use of cooked potato in various ways by Scottish and English bakers, and claims that substantial improvement is noted in the loaf when potato is included in the dough mix. He attributes the utility of the potato to the soluble nitrogenous material and dextrinized starch contributed to the dough and available as food for the yeast and its enzymes. The modern commercial baker, however, depends upon various manufactured flour improvers to get increased bread quality from his flour. These improvers possess the advantage of being available in a form very convenient for use. The only kind of potato employed appears to be a factory product in the form

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[2] *Chemist, Quaker Oats Company, Saskatoon.*

of potato flour, which has a limited use for special breads and pastries.

To ascertain whether the use of potato as a flour improver was justified and to determine its effect upon the relation between crude protein and baking strength, the following work was carried out.

Materials and Methods

The flours used in this study were all of commercial type and were milled from the 1930 Western Canada crop with the exception of No. 1, which was produced from Ontario soft winter wheat. Flour No. 2 was milled from a blend containing a high percentage of Garnet and showed a rather dark loaf color. Several millstream flours, No. 3, 9 and 10, were included to obtain information regarding the bleaching effect of potato upon unbleached flour and to furnish samples in the higher protein range. Sample No. 8 was a strong first clear and produced a dark colored loaf of inferior grain and texture. Samples No. 4 and 5 were first patent flours while No. 6 and 7 were second patents.

The baking method was as follows: the doughs were mixed by hand in earthenware bowls (height, $4\frac{1}{2}$ in.; diameter, 5 in.; thickness of wall, $\frac{1}{4}$ in.) and run in pairs at intervals of five minutes. The Blish standard method (2) was followed in regard to fermentation, proofing time and temperature. The formula used was: flour, 100 gm.; yeast, 3 gm.; sugar (sucrose), 2.5 gm.; salt, 2 gm.; distilled water, as required for proper consistency. The salt and sugar solutions were added from a 100-cc. burette, 10 cc. being required for each dough. The water was measured in a 50-cc. burette supplied by syphon from a 1000-cc. reservoir. One of each pair of doughs was checked for temperature subsequent to mixing, and warm water added to the reservoir if the dough temperature became lower than 29° C. The absorption was varied to suit the requirements of each flour. The yeast suspension was measured in a 10-cc. graduated cylinder. The salt and sugar solutions used, as well as the yeast suspension, were corrected for volume of solute. This method is called the simple or basic procedure.

A modification of the simple procedure was also used, including the addition of 1% diastatic malt and 0.001% $KBrO_3$ to the basic ingredients. The malt was introduced as a solution, 4 cc. containing 1 gm. diastatic malt and 3.3 cc. of water. The potassium bromate was added by means of a pipette from a stock solution, 1 gm. to 1000 cc. distilled water. This variation of the standard or basic procedure is called the improver method in this investigation. It has been extensively used by Larmour and MacLeod (11), Geddes (4), Geddes and Goulden (5), and Harris (6). The utility of this method lies in the fact that it tends to bring out the inherent possibilities of a flour, which might escape detection when the basic method alone is used. The action of these ingredients is also apparently analogous to that of those added in the treatment of flour in a commercial bakery, where flour improvers are in common use.

Results

Effect of Cooked Potato

The simple basic formula plus the required quantity of potato (liquid or

mashed) was used in determining the effect of the cooked potato upon the baking properties of the flours. This ingredient was prepared as follows: the potato water was drained from peeled and sliced potatoes after boiling until the potatoes were thoroughly cooked. The liquid was quite opalescent especially at first, and deposited material of a starchy nature upon standing in a cool place. When incorporated in dough this deposited matter apparently possessed greater beneficial properties than the original liquid. Centrifuging had no appreciable effect upon the bread improving properties and did not remove the opalescence of the liquid. The potato water was concentrated by boiling at atmospheric pressure without changing its effect, but as the liquid became more viscous with increasing evaporation the color darkened rapidly, with resultant detriment to the loaf color. The stimulative action however persisted. After several attempts the concentration of the liquid by evaporation was abandoned and cooked mashed potato substituted for the potato drainings.

For this purpose, the potatoes were first peeled and all blemishes removed. The peeled potatoes were then washed in water and sliced into small pieces, sufficient tap water being added to insure thorough cooking of the vegetable before the water evaporated sufficiently to cause burning. Approximately 25 min. was required to thoroughly cook the potato. The cooking was done in a galvanized vessel without a lid, to assist evaporation of the water, thus obviating loss of soluble material from the potato through draining off any residual liquid. The potato was then quite soft and easily disintegrated by stirring vigorously with a broad spatula. The wet mass was dried at a slow heat for approximately one hour with frequent stirrings. It was found that drying for too long a period caused hard, lumpy masses to form, which were very difficult to break up and came through the baking process practically unchanged. The moist potato also appeared to yield better results.

The potato water was found to contain 0.03 gm. of nitrogen per 25 cc. The mashed potato contained 0.41% of nitrogen and 0.82% of ash, and had a high moisture content. The percentage of moisture in the potato was not determined, owing to the difficulty encountered in drying to constant weight.

The inorganic residue left after ashing the potato at dull redness was found to be without any great influence upon the baking properties of the flour. A concentration as high as 5% was used, leaving the loaf volume quite unaffected, but slightly darkening the color to a greyish tinge. The texture also appeared to be somewhat coarsened. From this, it would appear that the effect of potato cannot be ascribed to the presence of inorganic salts carried into the dough with the potato.

Experiments with Raw, Uncooked Potato

A trial was made, using raw, uncooked potato in place of the boiled. The potato in this case was peeled and finely grated before adding the flour. No attempt was made to dry the wet mass on account of the possible effect of heat upon the proteins and starch. No improvement was noted except in color and here the change was very slight. It is evident that heating causes a

change in potato proteins and starches, rendering them more suitable as nutrient material in the dough. The enzymes in potato do not appear to have any great effect upon the baking process, as the heating necessary in thoroughly cooking the potato would tend to inactivate them. To settle this point definitely, a portion of the potato water was evaporated to a dark brown paste and heated to the burning point (120-125° C.), then the paste was used with the simple formula in baking several loaves. No diminution in the improving action of the material upon loaf volume was noted.

The baking score was computed in the following manner:

Loaf volume	×0.1		
Symmetry	×1.0	Maximum value	10
Grain of loaf	×1.0	Maximum value	10
Color	×1.0	Maximum value	20
Texture	×1.0	Maximum value	10

The sum of these individual scores was considered the baking value.

Effect of Potato Water

Table I shows the baking data obtained on two commercial flours using varying quantities of potato water from 0 to 50 cc. A regular increase in

TABLE I

BAKING DATA OBTAINED WITH TWO FLOURS WHEN INCREASING QUANTITIES OF POTATO WATER WERE INCLUDED IN THE BASIC FORMULA

Flour No. 11							Flour No. 12						
Potato water, cc.	Volume, cc.	Symmetry	Color	Grain	Texture	Score	Potato water, cc.	Volume, cc.	Symmetry	Color	Grain	Texture	Score
0	470	9	20	10	10	96	0	480	7	16	8.5	9	88
10	520	10	22	10	11	105	10	578	8.5	17	8	8	99
20	570	10.5	22	9.5	10.5	109	20	620	9	17.5	8	9	105
30	590	11	23	9.5	9.5	112	30	630	10.5	18	8	9	108
40	598	11	22	9	9	111	40	645	11	18.5	7	8	109
50	615	11	19	8.5	9	109	50	660	10.5	19.5	7	7.5	110

loaf volume is noted with increasing concentration of potato water. The symmetry and color also tend to improve although there is some evidence of falling-off in color after 30 cc. in one case. The grain and texture scores of the loaf decrease with increasing potato water.

Effect of Higher Potato Concentration

To determine the effect of higher potato concentration upon loaf volume and color, the mashed potato was employed in concentrations of 1 to 60% on 100 gm. of flour, and also with different proportions of potato and flour up to 50 gm. of each, using 100 gm. of combined flour and potato. The baking data thus obtained is shown in Table II. A regular increase in loaf volume is shown with increasing quantities of potato, and when the values are calculated on 100 gm. of total material, an increase in loaf volume over the basic results

TABLE II

BAKING DATA OBTAINED BY INCLUDING INCREASING PERCENTAGES OF COOKED MASHED POTATO IN THE BASIC FORMULA. CORRECTED TO 13.5% MOISTURE BASIS

% Potato	Loaf volume, cc.		Symmetry	Color	Grain	Texture	Scores calculated using *a* and *b* loaf volumes	
	a	*b*						
Flour No. 13								
0	490	490	8	21	9	9.5	96	96
1	505	500	9	20.5	8.5	9	97	97
2	520	509	10	21	8	9	100	99
5	545	519	10.5	23	6	7	101	98
10	580	527	10.5	24	6.5	7	106	101
15	620	539	10.5	24	4	5	105	97
20	645	537	10	25	3	3	105	95
25	642	513	9.5	26	3	3	96	83
30	695	535	10.5	26	3	2	111	95
40	735	525	10	26	2	2	113	92
50	755	503	9	25.5	2	2	114	89
Flour No. 14								
0	490	490	8	22	9.5	9	97	97
1	482	477	8	22.5	10	10	98	98
2	512	502	9	23	10	10	103	102
5	528	503	9.5	24	8	8	102	100
10	585	532	9.5	23	7	7	105	100
15	590	513	10	24	6.5	7	106	99
20	620	517	10	23.5	5	5	105	95
25	660	528	10	24.5	5	5	110	97
30	670	515	10	24	4.5	5	110	95
40	680	485	10	24.5	4	5.5	112	92
50	725	483	10	22	3	4	115	87
60	750	468	10	21.5	2	3	111	83

NOTE: *a = measured loaf volume on 100 gm. flour; b = loaf volume calculated on 100 gm. of total material.*

is evident up to at least 30% of potato concentration. This behavior is also shown in the case of the bakings with a total of 100 gm. of combined potato and flour. An increase over the basic loaf volumes is shown up to a flour-potato ratio of 70:30, corresponding to 42.8% potato to 100 gm. of flour. The color score of the loaf also increases with increasing quantities of potato, reaching a maximum at about 30% potato, when the larger loaves are considered. In the case of the test with a total of 100 gm. of the material, the color in one instance becomes darker at the 50-50 concentration, but with the other flour the color does not fall even at this point.

The loaves baked with 100 gm. flour plus potato show progressive decrease in grain and color after the initial treatment with potato, becoming very poor for the higher concentrations. The same trend is evident, possibly in a less marked degree, with the other bakings. The bread produced above concentrations of 10% of potato would probably be considered unsatisfactory for commercial purposes. It was noticed that the bread made with potato

remained fresh and moist much longer than the basic loaves kept under the same conditions.

TABLE III

BAKING DATA OBTAINED BY INCLUDING INCREASING RATIOS OF COOKED MASHED POTATO TO FLOUR IN THE BASIC FORMULA

Ratio flour/potato	Potato as per cent of flour	Loaf volume, cc.		Symmetry	Color	Grain	Texture	Score
		a	*b*					
Flour No. 13								
100 Flour 0 Potato	0	490	490	8	21	9	9.5	96
90 Flour 10 Potato	11.1	540	600	9.5	22	9	9.5	104
80 Flour 20 Potato	25.0	520	650	10	23	7	7	99
70 Flour 30 Potato	42.8	510	729	8	24	4	4	98
60 Flour 40 Potato	66.6	434	723	7	25	4	4	84
50 Flour 50 Potato	100.0	365	730	4	25	3	2	70
Flour No. 14								
100 Flour 0 Potato	0	490	490	8	22	9.5	9	97
90 Flour 10 Potato	11.1	540	600	10	24	8	8	104
80 Flour 20 Potato	25.0	550	687	10	23	7	7	102
70 Flour 30 Potato	42.8	520	743	9	24.5	6	6.5	88
60 Flour 40 Potato	66.6	460	767	7	25	5.5	6	89
50 Flour 50 Potato	100.0	372	744	3	22	3	3	68

NOTE: *a = measured loaf volume on 100 gm. material; b = loaf volume calculated on 100 gm. of flour.*

Effect of Potato on Gas Production and Retention of Fermenting Doughs

In view of the remarkable effect of cooked potato upon loaf volume, it was thought advisable to investigate the action of the vegetable on the gas-producing and gas-retaining power of fermenting doughs. A modification of the method suggested by Bailey and Johnson (1), and subsequently used by Johnson and Bailey (8), St. John and Bailey (12), St. John and Hatch (13), and Karacsonyi and Bailey (9), was employed. The procedure in the present

instance differed from the original method chiefly in that dough from 25 gm. of flour was taken for each determination and 100-cc. beakers were used inside of pint Mason jars to hold the dough, instead of glass cylinders perforated near the top and fitted tightly against the lid of the jar. The apparatus used was designed by Dr. R. K. Larmour, Chemistry Department, University of Saskatchewan, and proved very convenient for the purposes of the present investigation.

Three flours were included in this study, No. 2, 5 and a baker's patent milled from Western Canada wheat containing 13% of protein. The first run was made with flour No. 5 using doughs made with the simple formula alone, as a control, and with the basic plus 5, 10 and 20% of cooked potato. Two controls were run, one to measure the total amount of gas evolved from the fermenting dough, and the second to determine the volume of the dough itself. The other tests were made in duplicate, the dough as mixed being divided immediately after mixing into four equal parts. In this way two readings were obtained for each determination. In all, 14 determinations were made in each run.

TABLE IV

INCREASE IN VOLUME OF DOUGH, VOLUME OF CARBON DIOXIDE LOST FROM DOUGH, AND SUM OF THESE VOLUMES DETERMINED AT 30-MIN. INTERVALS FOR THREE HOURS

Controls			Control+5% potato			Control+10% potato			Control+20% potato		
Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough
13	13	0	12	12	0	12	12	0	12	12	0
67	62	5	57	50	7	58	56	2	51	56	−5
126	83	46	106	77	29	109	78	31	113	80	33
179	91	88	153	83	70	162	90	72	163	95	68
223	99	124	205	93	112	207	99	108	227	100	127
274	102	172	250	98	142	266	101	165	285	107	178
312	107	205	288	101	187	313	104	209	347	112	235

The first run was made over a period of three hours after mixing, readings being taken at ten-minute intervals. The results obtained were quite disappointing, as shown in Table IV. No definite increase in gas evolved or volume of the fermenting dough was evident when the treated doughs were compared with the controls. When baking a series of doughs both with and without potato it had been noted that the difference in size and rate of fermentation became most marked after panning, and increased as the doughs neared the oven. It accordingly seemed pertinent to make the measurements relating to gas formation after the usual three-hour fermentation period. The tests were made, therefore, after the two punches and at the time the doughs would ordinarily be panned. Decided differences between the various doughs were revealed by this method, as shown by the data in Table V.

TABLE V

INCREASE IN VOLUME OF DOUGH, VOLUME OF CARBON DIOXIDE LOST FROM DOUGH AND SUM OF THESE VOLUMES AS DETERMINED AT 30-MIN. INTERVALS FOR TWO HOURS, FOLLOWING THREE-HOUR FERMENTATION PERIOD

Controls			Control + 5% potato			Control + 10% potato			Control + 20% potato		
Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough
Flour No. 2											
19	14	5	22	18	4	19	26	−7	26	22	4
58	43	15	62	53	9	76	60	16	80	73	7
97	70	27	109	86	23	135	100	35	143	117	26
132	85	47	149	102	45	186	118	68	205	130	75
Flour No. 15											
12	14	−2	14	15	−1	17	15	2	12	11	1
52	52	0	64	63	1	74	70	4	84	81	3
89	82	7	117	110	7	134	111	23	161	133	28
122	106	16	166	125	41	190	123	67	230	170	60
Flour No. 5											
12	16	−4	19	17	2	18	13	5	11	11	0
56	47	9	57	50	7	61	52	9	66	60	6
90	74	16	96	81	15	113	92	21	129	110	19
122	96	26	133	101	32	156	122	34	186	139	47

Control + 30% potato		
Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough
17	15	2
87	80	7
163	132	31
244	153	91

Fig. 1 shows graphically the total gas evolved, the volume of the dough and the gas lost, over a 120-minute period. Flour No. 5 was used, with potato concentrations of 0, 5, 10, 20 and 30%. A progressive increase in gas evolved is shown in going from 0 to 30% potato, corresponding to a similar increase in dough volume. More gas is lost from the dough with increased dosage of potato, but this loss is more than compensated for by the increased gas production due to stimulation by the potato.

Fig. 2, 3 and 4 represent the data similarly obtained on the three flours.

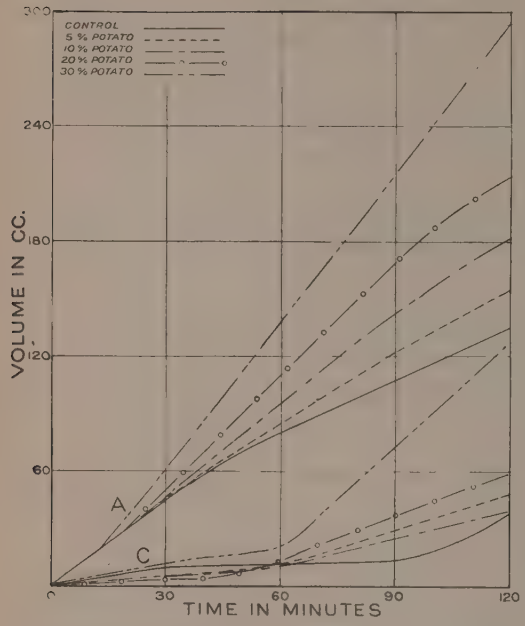

FIG. 1. *Changes in volume which occur in systems containing a fermenting flour dough, and fermenting dough plus potato. Curves A represent the sum of the increase in volume of the dough and the volume of carbon dioxide lost from the dough; Curves C, the volume of carbon dioxide lost from the dough.*

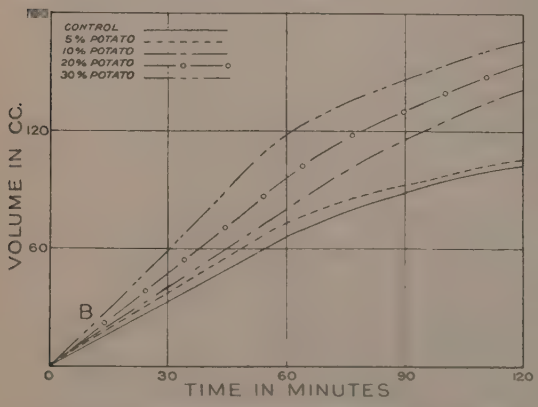

FIG. 2. *Changes in volume which occur in systems containing a fermenting flour dough, and fermenting dough plus potato. Curves B represent the increase in volume of the dough.*

Graph No. 2 shows the total gas evolved, No. 3 the volume of the fermenting dough, and No. 4 the gas lost from the doughs. The principal effect of the potato appears to be an enhanced evolution of gas. This effect increases with increasing potato concentration. The loss of gas also increases with the higher concentrations, but remains less than the increase in production. Flour No. 2 was weaker than flour No. 15, but evolved more gas when not treated with potato. The increase in volume of the dough was less, however, due to inability to retain the gas. It is probable that the soluble nitrogenous constituents of the potato, as well as the dextrinized starch, form a favorable nutrient medium for increased yeast propagation. This effect becomes more marked as the fermentation progresses, hence the doughs containing potato steadily show improvement over the untreated doughs during the later stages of fermentation and in the first few moments in the oven. During the first period, the potato material is probably not readily available for the yeast to act upon, but must be more thoroughly dissolved in the water present in the dough.

Comparison of Effect of Potato, and Malt and Bromate, on Flours of Varying Protein Content

It was considered advisable to ascertain the effect of various percentages of the prepared potato upon a series of flours embracing a wide protein range, comparing the results obtained with the values yielded from

the same series with the use of malt and bromate. Accordingly, a series of 10 flours, as already described, was baked by the simple basic formula and by the basic method plus 5, 20, and 40 gm. of cooked potato added to 100 gm. of flour. A further baking was also made using 50 gm. each of potato and flour. The improver formula as described was also included in a baking series.

The protein content of the flours and the loaf volumes obtained with the various bakings are shown in Table VI. The scores assigned to the loaves are contained in Table VII. Examining first Table VI, it will be noted that the higher protein flours show a surprising reaction to the higher concentrations of the potato, while all the flours exhibit substantial increases in loaf volume. The data would seem to show a general trend toward increasing loaf volume with increasing flour protein in each series of bakings, this tendency being less marked in the case of basic values. A striking similarity is evident between the action of all the doughs treated with potato and the malt and bromate baking. The loaf volumes obtained with the 50-50 blend are remarkably high when calculated on the basis of 100 gm. of flour, and show no evidence of decrease of baking strength with the large proportion of potato to flour.

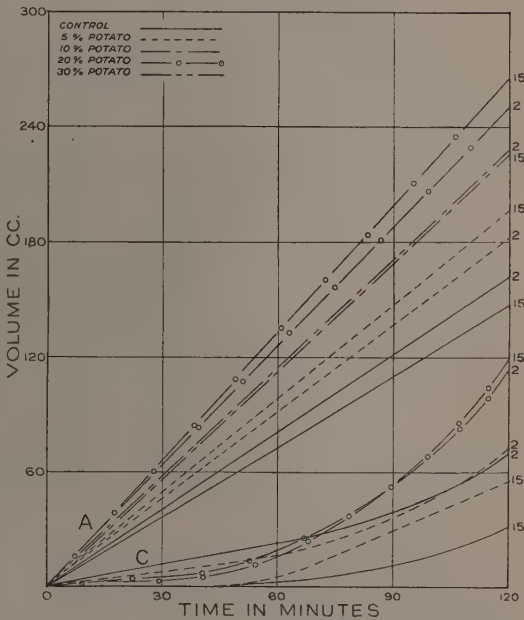

FIG. 3. *Changes in volume which occur in systems containing fermenting flour doughs and fermenting doughs plus potato. Curves A represent the sum of the increase in volume of the dough and the volume of carbon dioxide lost from the dough; Curves C, the volume of carbon dioxide lost from the dough.*

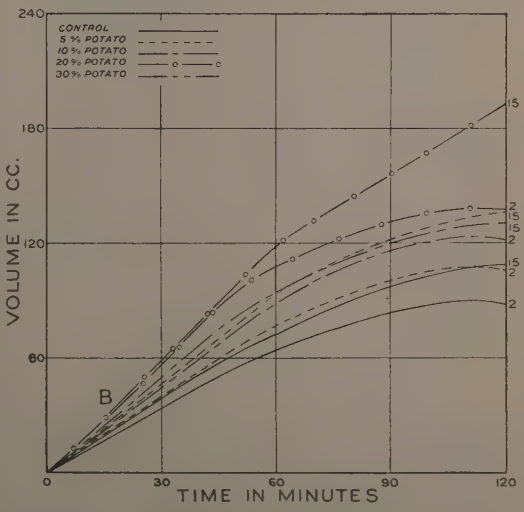

FIG. 4. *Changes in volume which occur in systems containing fermenting flour doughs and fermenting doughs plus potato. Curves B represent the increase in volume of the dough.*

TABLE VI

COMPARATIVE LOAF VOLUMES OBTAINED WITH BASIC FORMULA, WITH BASIC PLUS VARIOUS PERCENTAGES OF COOKED MASHED POTATO AND WITH IMPROVER FORMULA

No.	Protein %	Loaf volume, cc.					
		Concentration of potato, %				Flour, 50 gm.; potato, 50 gm.	Improver method, 1% malt+0.001%$KBrO_3$
		0	5	20	40		
1	7.8	370	380	420	475	420	410
2	10.7	450	450	560	610	590	535
3	11.3	420	478	542	640	570	465
4	11.8	490	532	560	665	700	585
5	12.2	480	530	595	640	750	550
6	13.3	500	545	577	690	800	610
7	13.6	475	530	615	660	740	640
8	17.4	510	560	630	720	780	645
9	17.8	610	652	703	840	900	800
10	18.8	520	600	690	825	960	810
Av.		482.5	525.7	589.2	676.5	721.0	605.0

The average loaf volume is highest for a high concentration of potato although the loaves were of very poor appearance and shape, were lacking in bloom and had a rather heavy soggy interior. These loaf volumes were calculated on the basis of 100 gm. of total material and the values obtained are shown in Table IX. It is seen from this table that the volumes tend to increase up to a concentration of 20% potato, falling at 40% to the basic values, as registered by the average results. The loaf volumes obtained with the improver, however, are larger than the corresponding potato results with one exception, flour No. 3 at 20%.

The baking scores as shown in Table VII reveal little change in color or symmetry with increasing concentration of potato. The grain and texture grow progressively poorer due to the loosening up of the loaf by the potato. The baking score is influenced by the loaf volume to such an extent that an increase is evident here with increase of potato. To obviate this difficulty, the scores were recalculated, using loaf volumes corrected to 100 gm. of total material. These results are shown in Table VIII. The largest single score in this table is yielded by the improver data while the largest increase in the potato treatments over the basic values is shown by the 5% concentration. The 40% treatments have the lowest scores, owing to the poor texture and grain of the loaves baked with this concentration of the vegetable.

Correlation constants were calculated between crude protein and the loaf volumes obtained by the different baking tests. These values are shown in Table X, with the points of minimum significance from the table of values at the 5% points, according to the number of pairs (3). These constants are all very significant, and show a decided relationship between protein and baking strength. The basic results would appear to be slightly less related to protein than the other baking data, but the difference is not significant. The cor-

TABLE VII

BAKING SCORES ASSIGNED LOAVES BAKED WITH BASIC FORMULA, WITH BASIC FORMULA PLUS POTATO AND WITH BASIC FORMULA PLUS MALT AND BROMATE

Flour No.	1	2	3	4	5	6	7	8	9	10	Av.
Color of loaf											
B	15	9	11	19	21	17	19	8	13	8	14.0
5%	14.5	10	11.5	21	21.5	18	19	8.5	14	9	14.7
20%	15	8	12	20	20.5	18.5	20	9	14.5	9.5	14.7
40%	14	8.5	13	20	20	17.5	19	7	14	8	15.1
1	12	10	10	18	16	17	19	9	15	11	13.7
Symmetry of loaf											
B	4	7	5	9	7	8.5	7	8	9.5	9	7.4
5%	4.5	7.5	6	10	8.5	9	8.5	8.5	10.5	9	8.2
20%	5	9.5	7	8	9	9.5	9	6.5	10.5	9	7.5
40%	4	7	8	9	9	10	9.5	6.5	10.5	9	4.0
1	3	8	4	9	7	10	10	9	11	11	8.2
Grain of loaf											
B	6	6	9	9	10	8	8	5	9.5	7	7.7
5%	5	7	8.5	8	9	7	8.5	5	6	6	7.0
20%	4	4	5	6	6.5	6.5	7	4.5	8	7	5.8
40%	2	2	4	5.5	4	5	5	5	7.5	2	4.2
1	6	7	6	7	8	6	8.5	5	7	7	6.7
Texture of loaf											
B	4	6.5	9	9	10	8	7.5	6	8	7	7.5
5%	4	7	9	8.5	10	7.5	8	5.5	7	5	7.1
20%	2	5	5.5	8	9	7.5	8.5	4.5	8	4.5	6.2
40%	1	4	4	7.5	5	8	6	4.5	8	4	5.2
1	4	5	7	6	8	5	7	6	6	7	6.1
Score											
B	66	73	76	95	96	91	89	101	101	83	84.5
5%	66	76	83	101	102	96	97	83	103	89	90
20%	68	82	84	98	104	100	106	87	111	99	94
40%	68	82	93	108	102	109	105	95	114	105	98
1	66	93	73	98	94	89	108	93	119	117	95

relations calculated from the bakings with potato are very similar to the values yielded by those with the improver, and would seem to substantiate the view that potato functions in much the same manner as other, more widely known, flour improvers. Crude protein of flour is shown to be as important in relation to loaf volume when potato is included in the baking formula as when other flour stimulators are used.

These results appear to justify the use of cooked potato in various forms in bread making, as it produces larger loaves of improved color. The improve-

TABLE VIII

BAKING SCORES CORRECTED TO 100 GM. OF MATERIAL

No.	Basic method	Concentrations of potato, %			Improver method
		5	20	40	
1	66	64	64	55	66
2	73	74	77	65	93
3	76	80	79	75	73
4	95	98	93	89	98
5	96	99	99	84	94
6	91	93	94	79	89
7	89	94	100	87	108
8	78	81	82	74	93
9	101	100	105	100	119
10	83	86	93	82	117
Av.	85	87	89	79	95

TABLE IX

LOAF VOLUMES CORRECTED TO 100 GM. OF TOTAL MATERIAL

No.	Loaf volume, cc.					
	Basic method	Concentration of potato, %			Flour, 50 gm.; potato, 50 gm.	Improver method
		5	20	40		
1	370	362	382	340	210	410
2	450	428	509	436	295	535
3	420	455	493	457	285	465
4	490	507	509	475	350	585
5	480	505	540	457	375	550
6	500	519	525	493	400	610
7	475	505	560	472	370	640
8	510	533	573	514	390	645
9	610	621	640	600	450	800
10	520	571	627	590	480	810
Av.	482.5	500.6	535.8	483.4		605

TABLE X

CORRELATION CONSTANTS CALCULATED BETWEEN CRUDE FLOUR PROTEIN AND LOAF VOLUMES OBTAINED BY INCLUDING VARIOUS QUANTITIES OF POTATO IN THE BAKING FORMULA

Baking formula	Correlation constant
Basic method	+ .8479
Basic method + 5% Potato	+ .9017
Basic method + 20% Potato	+ .9342
Basic method + 40% Potato	+ .9442
Basic method + 50 gm. flour and 50 gm. potato	+ .9504
Basic method + 1% diastatic malt and 0.001% $KBrO_3$	+ .9335
Value at 5% point	+ .6319

ment in volume seems to extend to a high concentration of potato, but this gain is offset at these proportions by very poor grain and texture of loaf. The color also tends to fall off when the potato content is high. It certainly is not feasible in view of the extensive demand for a close, even-textured loaf, to replace a substantial portion of the flour by potato.

Conclusions and Summary

1. Cooked potato material has a beneficial effect upon doughs. This effect is especially noticeable in regard to loaf volume and color, and increases with increasing concentration of potato material. At high concentrations, the texture and grain of the loaf are adversely affected. The keeping qualities of the bread appear to be improved.

2. The cooked potato appears to function as a stimulant to the gas production, and while more gas is lost from the dough as compared with doughs made without potato, this loss is less than the gain in gas evolved. The net result is therefore a gain in dough volume. These effects increase as more potato is added to the dough. Strong flours are apparently better able to retain the gas produced than are weaker flours.

3. The beneficial effect of potato upon bread appears to be due to the soluble nitrogenous matter and starch contributed. No noticeable effect was produced by raw, uncooked potato or potato ash. Heating the potato had no effect upon the improver action, and as the potato enzymes would be inactivated by this treatment, the action is not due to enzymes.

4. Loaf volume is significantly related to crude flour protein in all the bakings made with the concentrations of potato used in this study. In this particular, potato corresponds to the action of malt and bromate. Flour protein would therefore be an important factor when cooked potato is used to supplant the usual improvers in the baking formula.

Acknowledgment

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STUDIES ON BROWNING ROOT ROT OF CEREALS

II. SOME PARASITIC SPECIES OF PYTHIUM AND THEIR RELATION TO THE DISEASE[1]

By T. C. Vanterpool[2] and J. H. L. Truscott[3]

Abstract

Evidence is presented which shows that browning root rot of cereals is caused primarily by species of *Pythium* the most important of which are *P. arrhenomanes* Drechsler var. *canadensis* n. var. and *P. volutum* n. sp. Soil conditions, especially those following summerfallow, and seasonal climatic factors also play a necessary role. Under experimental conditions *Pythium* injury to cereals manifests itself as an embryo rot or as pre-emergence killing of the seedlings, as post-emergence blighting, or as retarded development throughout the life of the plant, due to the impairment of the root system especially during the seedling stage. Both the spring and winter wheats are susceptible.

The isolation and inoculation methods found convenient in the study of the problem are outlined. *P. arrhenomanes* var. *canadensis* is widely distributed over the province, whereas *P. volutum* appears to be more limited in its range. Specific diagnoses of these two species and a discussion of their taxonomy are given. Other less aggressive species of *Pythium* undoubtedly contribute to the disease complex.

Comparative experiments show that *Pythium* injury to wheat may be as severe as that caused by *Ophiobolus graminis* or by *Helminthosporium sativum*. In general, *P. arrhenomanes* var. *canadensis*, the Louisiana sugar-cane *Pythium* and *P. arrhenomanes* are similar in their degree and range of parasitism, whereas *P. volutum* shows marked differences. Experimental evidence obtained under controlled conditions indicates that the damage caused to young wheat plants by *P. arrhenomanes* var. *canadensis* increases with both increasing soil temperatures and soil moistures. No correlation has been found between the hydrogen ion concentration of the soil and the distribution of the disease. Both *P. arrhenomanes* var. *canadensis* and *P. volutum* will grow in nutrient solutions with a lower pH value than that of any prairie soil tested, but optimum growth for both species occurs at neutrality. No conclusive results have as yet been obtained as to the effects of various fertilizers on the disease under artificial conditions.

Introduction

In recent years much attention has been given to the study of the relation of species of *Pythium* to plant diseases and to root rots in particular. Investigations on the association of *Pythium* species with root rot of cereals in Saskatchewan have revealed an interesting mycological and pathological problem in need of immediate attention. In an earlier paper by Vanterpool and Ledingham (25), it was considered from experimental evidence in the greenhouse that certain species of *Pythium* were the primary causal factor in browning root rot of cereals. The present contribution deals with a study of some of these parasitic species, their relationships to the disease, and attempts to elucidate the conditioning or environmental factors which predispose the host plants to attack. The many striking points of similarity between this root rot of cereals and *Pythium* root rot of sugar cane and corn in the United States, Hawaii, the Philippines and elsewhere, have necessitated the inclusion of comparative studies in this paper. Some recent field data on the disease are also included.

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General Observations

For a general description of browning root-rot disease of cereals reference should be made to the first paper of this series (25), as the following observations are, for the most part, supplementary to facts recorded there.

Field studies made during the past two summers have confirmed previous impressions that the occurrence of the disease, especially on wheat, is very closely related to the soil conditions brought about by the common practice of summerfallowing, as well as to seasonal climatic conditions. Several cases have been brought to our notice in which the yield of the wheat crop following summerfallow was about equal to or less than the yield of the second or stubble wheat crop on the same land. This was attributed to browning root rot in the fallow crop and its absence in the stubble crop. When, moreover, such infested land is again thoroughly fallowed and resown to wheat, browning root rot is very likely to reappear should weather conditions be suitable. Obviously, the parasitic fungi are present in the soil and what is essential for the appearance of the disease is a set of environmental factors acting on the host, on the parasite, or on both. Browning root rot was not very prevalent during the 1930 and 1931 growing seasons, due probably to the extremely dry conditions which prevailed during the spring of both years. However, throughout the north-central, and northeastern parts of the province, where the rainfall was normal or above normal, there were many scattered localities with severe cases of the disease. The root rot is most common on the brown soil of the Regina Clay type and on the black clay-loam soils of the park-land region, but it is occasionally found on soil of light texture, thereby indicating that soil type is not a limiting factor. Cases of the disease have been found on incipient podsol in the Birch Hills area. During 1930, wheat plants affected with browning root rot were received from Manitoba by Dr. P. M. Simmonds of the Dominion Laboratory of Plant Pathology, Saskatoon. In the same year Robertson (18) found the disease attacking wheat and oats in Alberta. These cases constitute the first reports of the disease outside of Saskatchewan.

The strong drying winds of the springs of 1930 and 1931 with their consequent soil drifting and mechanical damage unquestionably interfered with field diagnosis of the disease, as spots which ordinarily would have been bronzed by browning root rot, and the healthy green areas, alike showed the effects of "blowing." In many instances microscopical examination of wheat seedlings from such areas revealed abundant *Pythium* oospores in brown, flaccid root tips, both so characteristic of browning-affected plants. This masking may, in part, have accounted for the reported absence of browning root rot in many localities.

The first symptoms of the root rot were noticed during the first week in June when the wheat seedlings were about five to six weeks old. This agrees with its appearance in former years and suggests that there might be, under certain conditions, a *critical period* in the growth of the seedlings when their resistance to the attack of root parasites is lowered. That the plants frequently recover, although delayed in maturing, is further evidence in support of this supposition.

In one locality where browning is common a summerfallow wheat crop sown two weeks later than usual still contracted the disease, which, of course, further tended to delay maturity and render the crop more liable to rust infection and frost damage.

Cases were again reported where the disease failed to reappear on the wheat in old straw-stack spots after summerfallowing, although adjoining patches were affected. These facts suggest that the crop residue in the stubble crop possibly acts in some way as a soil amendment inhibiting attack from species of *Pythium*, in spots which were diseased in the previous year's fallow crop. The contradictory evidence regarding farmyard manure as an amendment for the trouble may perhaps be explained on the basis of its straw content.

During the spring of 1930 an examination of some sickly winter-wheat plants in the experimental plots at the University revealed the presence of *Pythium* oospores in brown root-tip lesions. The plants recovered, but showed signs of impaired vitality for the remainder of the season. It seems reasonable to suppose from this that *Pythium* may sometimes be a factor in the root-rot complex of winter wheat in many parts of the winter-wheat areas on this continent. Isolations from these winter-wheat plants several weeks later yielded only saprophytic species of *Pythium* or at most only very weak parasites. Rarely are parasitic species obtained when isolations from spring wheat affected with browning root rot are attempted in late July or early August. Therefore, to obtain the correct relationship of various species of *Pythium* to browning, periodic isolations should be made beginning one or two weeks before the first indications of injury appear, and continuing until recovery is apparent. It has been the experience of the authors that the parasitic species are vegetatively active at an early date, the duration of this activity depending on seasonal climatic conditions. Consequently, isolations made after the active parasites have formed their sexual spores will, in the large majority of instances, yield only fungi playing a secondary role. Unless definite information is available on the present and past conditions of the crop, on climatic and soil conditions, and perhaps other factors such as previous crops, mere lists of the fungous flora from diseased roots will be of little significance. R. D. Rands (17) in the United States, and C. W. Carpenter (1) in Hawaii, have had much the same experience with regard to *Pythium* root rot of sugar cane.

Recent Literature

A serious new disease of maize caused by a *Pythium* of the *gracile* group, and quite distinct from the root rot of maize caused by *Pythium arrhenomanes* Drech. in the United States (*cf.* 12) was reported from Italy by Curzi, in 1929 (3). The specific identity of this fungus was not given. From the same country in the following year Petri (16) described a species of *Pythium* as the cause of a disease of the basal portion of wheat plants in early summer. The *Pythium* disease of wheat in Saskatchewan on the contrary is confined almost entirely to the root system and never have lesions been found on the first and second internodes as illustrated in Petri's paper. Petri did not assign

his fungus to a definite species. Roldan (19) in 1930, published a note on the occurrence of *Pythium* root-rot disease of maize and sugar cane in the Philippine Islands. Whether one or two species of *Pythium* are concerned is not clear. Rice is another cereal which has been known for some time to be affected with a *Pythium* root rot in the Dutch East Indies (13). Sideris (21, 22) has recently reported nine species of *Pythium* (of the Nematosporangium group) which were obtained from diseased roots of *Ananas sativus* as being aggressive root parasites of *Triticum vulgare* and *Zea mays*. The experiments were presumably conducted under artificial conditions. The identification of many of these species which are morphologically very similar is unfortunately based on cultural differences on a variety of plant media some of which (and among them the most important, papaya agar) are unobtainable in temperate climates. Some justification may be found for this procedure, but it unquestionably makes it more and more difficult for workers who have obtained one or more of these morphologically similar species to know definitely what forms they are working with. The accurate determination of the identity of a species would entail an enormous amount of comparative culture work, and would have to be undertaken by one or two authorities on the group. The most common Saskatchewan wheat *Pythium* is morphologically very similar to several species described by Sideris, but whether it is identical with any one of them cannot definitely be determined from the descriptions alone.

Methods of Isolation

The usual method of placing necrotic root tissue on poured agar plates was found to be unsatisfactory for the isolation of parasitic species of *Pythium* or of other parasitic Phycomycetes. Fresh material obtained directly from a field showing early symptoms of browning, or from young plants grown in infested soil in the greenhouse, proved to be best for securing the desired forms. The field material was dug up with the aid of a trowel, care being taken to secure a large lump of adhering soil and not to damage the root system unduly; this was then placed in a covered can for conveyance to the laboratory. Young root tips were selected and after being examined for internal mycelium were kept in sterile water for one or two days; they were then placed on non-acidified water agar or cornmeal agar plates. As the mycelium spread out over the agar, hyphal-tip cultures of fungi of the desired type could be secured. Similar methods of isolation are described at some length by Drechsler (6), and in shorter form by Vanterpool and Ledingham (25). Occasionally, it was possible to isolate parasitic species of *Pythium* from browning root tips which had overwintered under field conditions.

Preliminary Experiments on Parasitism

With the pure cultures thus obtained a very convenient laboratory experiment for separating the parasitic and non-parasitic forms was then conducted. Apparently sterile wheat seedlings, with plumules and primary roots varying from one-half to three centimetres in length and outwardly free from seed-borne organisms, were selected from a moist-chamber allotment and transferred to

sterile 125-cc. Erlenmeyer flasks, each containing a piece of filter paper 4 to 5 cm. in diameter, and 5 cc. of sterile tap water. The flasks were then inoculated in duplicate with two-day-old agar cultures of each of the isolation forms to be tested and placed on the laboratory table. Uninoculated flasks were kept as controls. In the course of one to three days the pathogenic forms produced a stunting and a yellow to dark brown discoloration of the roots with consequent dwarfing of the shoots. These forms, together with any which may have caused a dwarfing without obvious discoloration of the roots, were then further tested for their parasitic tendencies on the various cereals in pot experiments in the greenhouse. Prolonged use of this preliminary flask method has convinced the authors that both aggressively parasitic species and species which later prove to be only weakly parasitic in pots are readily detected in the flasks. It is an accurate preliminary indicator of parasitism as well as being a time saver.

For the greenhouse parasitism tests the various fungi were grown on a sterile, moist, oat-barley mixture for seven to ten days, after which 10 gm. of inoculum to each 6-in. pot was incorporated with the top three inches of steam-sterilized field soil to which about one-sixth sand had been added. To one series of controls, uninoculated oat-barley medium was added in a similar manner; the other series of controls was left untreated. This medium produced no appreciable toxic effects on the control plants under these conditions, which is in accord with the findings of McKinney and Davis (14). Sand-cornmeal inoculum medium was often found suitable for forms which do not grow well on the oat-barley mixture. The various parasitic species of *Pythium* which the authors have studied grow very poorly or not at all on sterile, moist, crushed oat hulls which are recommended by various workers for other root-rot

FIG. 1. *A, portion of a wheat plant showing the dark root-tip lesions characteristic of Pythium injury, collected from an infested field. B, wheat plants, four weeks old, grown in pots of steam-sterilized soil artificially inoculated with a pathogenic species of Pythium.*

parasites such as *Helminthosporium sativum*, *Ophiobolus graminis*, or *Fusarium* species. The seed grain was treated with mercuric chloride (1:1000) for 10 min., then washed thoroughly in water, and sown immediately. At the conclusion of the experiments the amount of damage was estimated on the percentage of seedlings in the final stand, their oven-dried weight, their average height, and the conditions of their root systems, and was rated as trace, slight, moderate or severe. The foregoing method was the one generally used in the pot studies reported in this paper. Any departure from this method is given for any specific experiment.

By comparative cultural studies the large number of parasitic forms were grouped into species; a few of the most parasitic strains of each species were then selected for more extensive studies on their relationships to the disease under controlled environmental conditions. By using the above methods it was relatively easy to demonstrate the vigorous parasitism of several species of *Pythium* isolated from decaying wheat roots (Fig. 1, B), but it is a difficult matter to determine their exact relationships to the disease under crop-culture conditions.

Distribution of the Pathogenic Species

As already mentioned, the frequency of isolation of any particular parasitic species in a given season does not necessarily give a true representation of its distribution, because conditions favoring the active growth of these fungi, during which time they yield most readily to isolation, vary considerably in duration over wide areas. However, isolations made from fresh field material, from greenhouse material grown in infested soil, and in a few instances from browning root tips collected from over-wintered stubble material, have yielded vigorously parasitic species of *Pythium* from Alameda, Regina, Moose Jaw, Saskatoon, Vanscoy, Rosetown, Rosthern, Scott, North Battleford, Prince Albert, Prudhomme and Tisdale. The disease is by no means confined to these localities; the list, on the other hand, gives some idea of the wide distribution of the root rot over Saskatchewan. The *Pythium* flora obtained from wheat roots may be divided into three groups as follows:

1. Those forms which are actively parasitic on wheat. In this group are included two species showing vigorous parasitism and at least three species with moderate, though aggressive, parasitic ability. One of these latter species was obtained from wheat roots grown in virgin prairie soil.

2. Those forms weakly parasitic on wheat even under optimum conditions for infection; and,

3. Saprophytic forms.

One or more of the parasitic species in group 1 have been obtained from all of the numerous browning root-rot fields which were given careful study. In the large majority of these fields *Pythium arrhenomanes* var. *canadensis* n. var. appears to be the chief form concerned in the damage, yet in several districts the root-rot situation is complicated by the presence of the other actively parasitic form, *Pythium volutum*, n. sp., and by various other forms, all of which are capable of strong parasitism. In only one locality where browning root rot is

severe have the authors failed to obtain *P. arrhenomanes* var. *canadensis* in their isolations. From this locality, however, *P. volutum* has been readily obtained on many occasions. *P. volutum* and *P. arrhenomanes* var. *canadensis* show approximately the same parasitism on wheat, but *P. volutum* is a much more active oat parasite (Fig. 6 and 7). Its strong parasitism on oats readily separates it from the other species. Although browning root rot can be produced experimentally by at least four species of *Pythium*, in most cases under field conditions, it is probably due to two or more of these species jointly. Cultural and etiological studies of *P. arrhenomanes* var. *canadensis* and *P. volutum*, the two species considered to be of primary importance in the browning root-rot situation, will be described at some length in this paper.

Pathogenic Species Considered

I. *Pythium arrhenomanes* var. *canadensis* n. var.

No reliable means of identifying the various species of *Pythium* from oospore characters within the cereal host tissue has been discovered. Only rarely have reticulate *Pythium* oospores been observed in wheat roots and it is believed that they are of little or no importance pathologically. It is, however, easy to distinguish all *Pythium* oospores from the resting spores of *Asterocystis radicis*

FIG. 2. *Pythium arrhenomanes var. canadensis. A, a lobulate sporangium showing both intra- and extra-matrical development; a portion of the tube of discharge is also shown. B, a sporangium in an epidermal cell of a root immediately before discharging its contents through the evacuation tube into a vesicle at its apex; rd, refractive dome just before being blown out into a vesicle. C, the protoplasmic contents of the vesicle have differentiated into zoospores. D, empty sporangia with portions of their evacuation tubes. E, simple toruloid sporangia. Drawn with the aid of a camera lucida.* ×700.

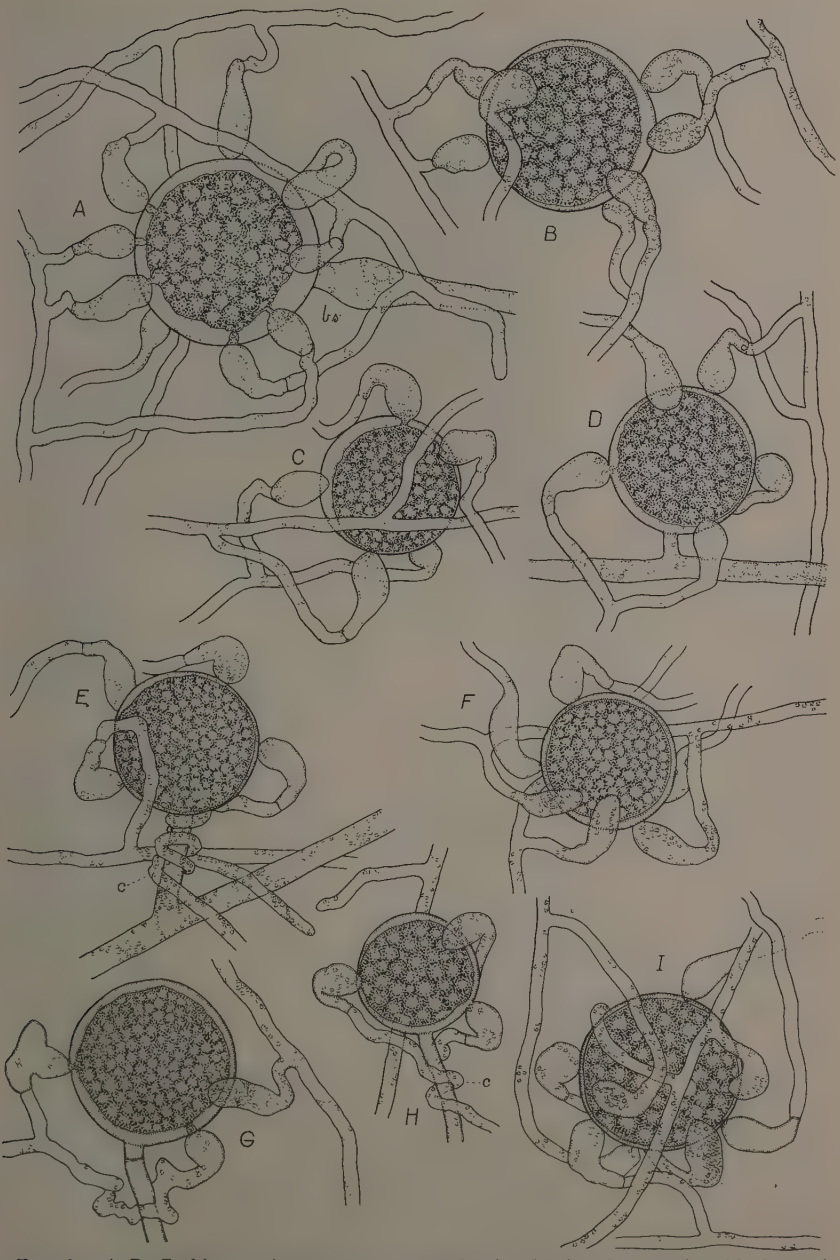


FIG. 3. *A–D, Pythium arrhenomanes var. canadensis, showing the oogonia with the crook-necked antheridia arising from passing hyphae; at A, bs, is a bulbous swelling which is occasionally observed on the oogonial stalk. E–I, Pythium volutum, showing typical oogonia and antheridia; at E, c, and H, c, the antheridial hyphae are shown enlacing the oogonial stalk. Drawn with the aid of a camera lucida.* ×700.

(26). *P. arrhenomanes* var. *canadensis* when growing actively in the host tissues produces lobulate sporangia which at times may fill the whole cell cavity. On this basis it can be distinguished from *P. volutum* which only very rarely produces small toruloid buds and never swollen digitate complexes. Under aquatic conditions the sporangia of *P. arrhenomanes* var. *canadensis* communicate with the outside by means of discharge tubes of which there may be one or more to each lobulate complex, though doubtless the lobulations are separated by septa into compartments, each with its own discharge tube (Fig. 2, A, B, and D). It is difficult to observe these septa before discharge of the sporangial contents. The placing of sporangia-containing roots in water which is changed constantly is usually the best means of initiating zoospore discharge (Fig. 2, B and D). The sporangia, instead of germinating by means of zoospores, often produce one or more germ tubes which develop into ordinary mycelium, in which case they may be said to function as "conidia." Various intermediate abnormal types of sporangial germination have been observed.

In culture, the morphology of the fungus has been studied principally on cornmeal and carrot-cornmeal agars and on steam-sterilized wheat roots in a small quantity of water in 125-cc. Erlenmeyer flasks. It was found that under these cultural conditions there is a greater tendency for the fungus to produce sexual bodies than asexual sporangia, and this holds over a wide range of temperatures. Although oogonia and antheridia are formed abundantly in culture (Fig. 3, A-D and Plate I, 7 and 8), maturation of the oospores occurs only in a small percentage of cases. Instead, the potential oogonia either become emptied of their contents or they may put out several germ tubes (Plate I, 6); this latter phenomenon may happen even after the antheridia have been applied to the oogonia (*cf.* 16). It has been ascertained that oospores formed in wheat roots average 2 to 3 μ smaller in diameter than oospores formed in agar media. Hence the importance of stating specifically the substratum of the oospores of which measurements were taken.

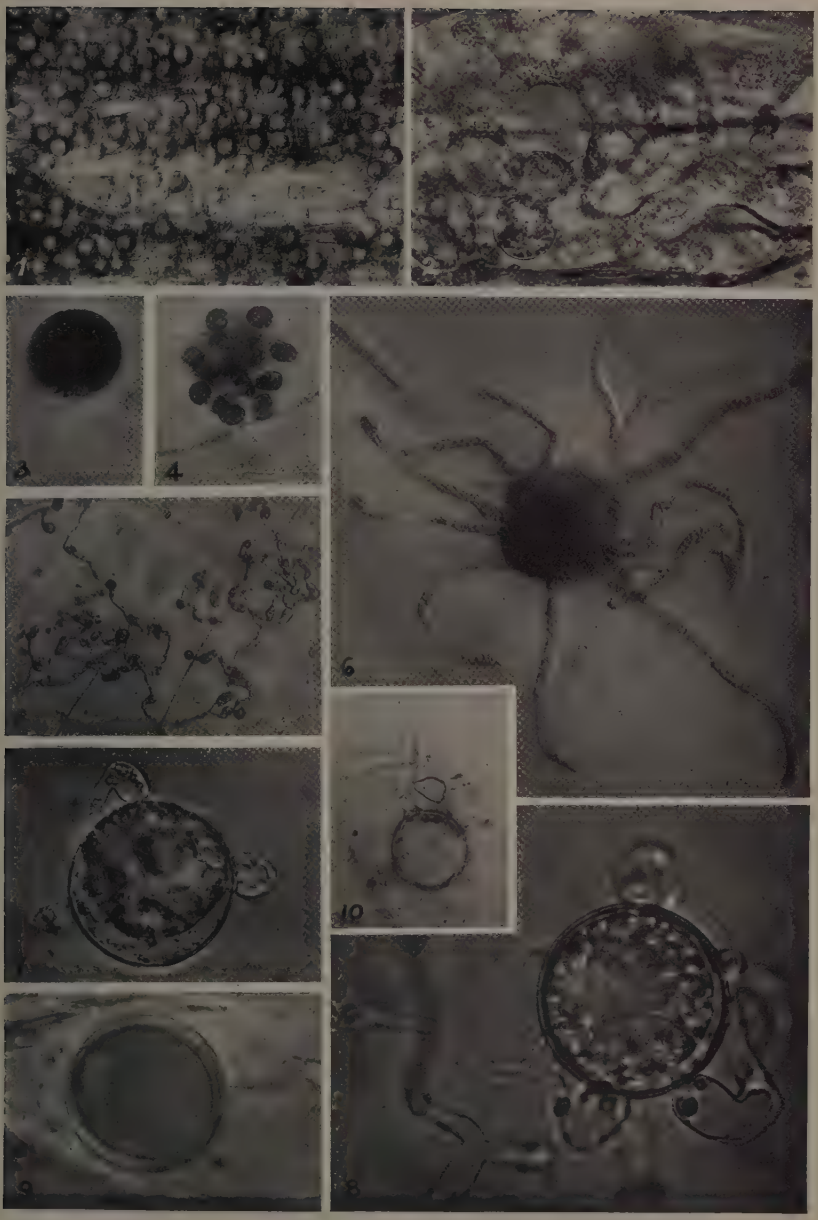
Diagnosis

Pythium arrhenomanes var. *canadensis* n. var.

Mycelium finely granular, lustrous and non-septate when young, clear and vacuolate with occasional cross walls when old, branching irregular, aerial development slight, radial growth rate on agar approximately 28 mm. in 24 hr. at 22° C.; optimum temperature for vegetative growth between 30° and 35° C.; lateral falcate outgrowths or appressoria commonly formed in agar plates; in host tissue mainly intracellular, extramatrical development under aquatic conditions.

Sporangia consisting of swollen lobulate elements ranging from toruloid lateral buds to compound complexes, terminal or intercalary, provided with a discharge tube 3 to 5 μ in diameter and up to 150 μ or more in length, seldom formed on solid media but often in liquid media; zoospores from 3 to more than 50 in a vesicle, bi-ciliate, deeply grooved at the hilum region, broad in ventral view, approximately 12 μ when rounded up, monoplanetic, germination by a single germ tube.

Oogonia spherical to subspherical, terminal or rarely intercalary, the



Pythium arrhenomanes var. canadensis. FIG. 1. *Oospores as seen in a typical necrotic root-tip lesion, ×150.* FIG. 2. *Lobulate sporangia in the cortex of a wheat root, ×600.* FIG. 3. *A vesicle after complete discharge of sporangial contents, ×600; stained with lacto-phenol-carbol fuchsin.* FIG. 4. *Zoospores just before discharge, ×600; stained with lacto-phenol-carbol fuchsin.* FIG. 5. *Appressoria formed in agar, ×200.* FIG. 6. *A potential oogonium which has produced several germ tubes, ×400.* FIG. 7. *Oogonia and antheridia on 3-day carrot-cornmeal agar, ×600.* FIG. 8. *Same as Fig. 7, ×900.* FIG. 9. *An oospore more or less completely filling the oogonium in an artificially inoculated wheat plant, ×900.* FIG. 10. *An oospore of unknown species from field material germinating by a germ tube, ×600.*

majority ranging from 27 to 40 μ in diameter (average 33 μ) on carrot-cornmeal agar on which they form in two to three days; in liquid culture a bulbous swelling occasionally forms on the oogonial stalk near the oogonium. Antheridia characteristically crook-necked, kneed or clavate, making narrow apical contact with the oogonium, delimited by a single septum, usually 3 to 6 but as many as 12 may be counted, commonly arising from neighboring hyphae, as many as four from one hypha.

Oospores smooth, spherical to subspherical, light brown, usually completely filling the oogonium, average diameter 31 μ, with double wall (2 μ), central globule (17.5 μ), and oblate refringent spot; occasionally oblong oospores with two reserve globules are found.

Cause of a root rot of *Triticum aestivum L.* in Saskatchewan. Also shown to be an aggressive root parasite of *Avena sativa L.*, *Hordeum sativum L.*, *Secale cereale L.*, and *Zea mays L.* Type culture from diseased roots of *Triticum aestivum L.*, Saskatchewan, 1929.

II. *Pythium volutum* n. sp.

Isolations of this species have repeatedly been obtained from the Tisdale district in the park land and the Regina plains in the south. The two strains from these respective regions show consistent minor cultural differences, but these are not considered sufficiently important to warrant their separation into different varieties, though they may be regarded as biologic strains.

On ordinary solid media or agar containing small pieces of grated carrot or wheat roots, no lobulate sporangia have ever been observed. These are rarely ever found in culture; they have been observed on a few occasions in wheat-root-water-culture flasks as lateral outgrowths or buds from the hyphae. Zoospore discharge does not readily occur, only three cases having been observed outside infected wheat roots. In none of these was it possible to distinguish the empty elements within the roots which gave rise to the vesicles, so that the statement that it is the lateral lobulations in this form which function as sporangia is actually based on analogy. On the other hand, typical sphero-sporangia have never been observed. Old agar cultures are dull brown and granular owing mainly to the presence of oospores and numerous empty sterile oogonia. The antheridia arise from neighboring hyphae and commonly coil characteristically around the oogonial stalk (Fig. 3, E and H, Plate II, 1) or, less frequently, around an adjacent hypha. Very rarely, an antheridium has been observed arising from the oogonial stalk. In artificially infected wheat roots in small flasks, oogonia have repeatedly been observed with antheridia in adjoining cells, but applied so that the tip of the antheridium passes through the host cell wall before making direct contact with the oogonium (Plate II, 3). Whether the antheridium forced or dissolved its way through the wall, or whether it passed through a mechanical opening already present, is not known.

Diagnosis

Pythium volutum n. sp.

Mycelium non-septate, lustrous, with an aerial tendency in culture, radial

growth on agar of approximately 16 mm. in 24 hr. at 22° C.; appressoria consisting of lateral falcate structures with rounded ends; mostly intracellular in host tissue.

Sporangia consisting of small lobulations or toruloid buds formed only rarely in aqueous culture; discharge tube usually about 50 μ long and 3-4 μ wide; zoospores biflagellate, bean-shaped, about 10-14 μ.

Oogonia smooth, subspherical, dark brown, terminal on short side stalks or rarely intercalary, formed copiously in culture but a large percentage remain sterile, average diameter 30 μ; antheridia 3 to 10 to each oogonium, crook-necked, sometimes curved or even straight, with narrow apical contact, usually arising from adjacent hyphae each of which supplies one to four antheridia, or more rarely arising from oogonial stalk; antheridial branches commonly entwine about the oogonial stalk in liquid media, but less frequently on solid media.

Oospores smooth, spherical to oblong, usually free within the oogonium, average diameter 27.7 μ, central globule 14.2 μ, refringent spot 8.5×2.2 μ, oospore wall 2.0 μ. Oblong oospores (average 36.9μ×19.2 μ) with two reserve globules are sometimes present in the host cells.

Causes a root rot of *Triticum aestivum L.* and *Avena sativa L.* in Saskatchewan. Also an aggressive root parasite of *Hordeum sativum L.*, *Secale cereale L.* and *Zea mays L.* when artificially inoculated. Type culture from diseased roots of *Triticum aestivum L.*, Tisdale, Saskatchewan, 1929.

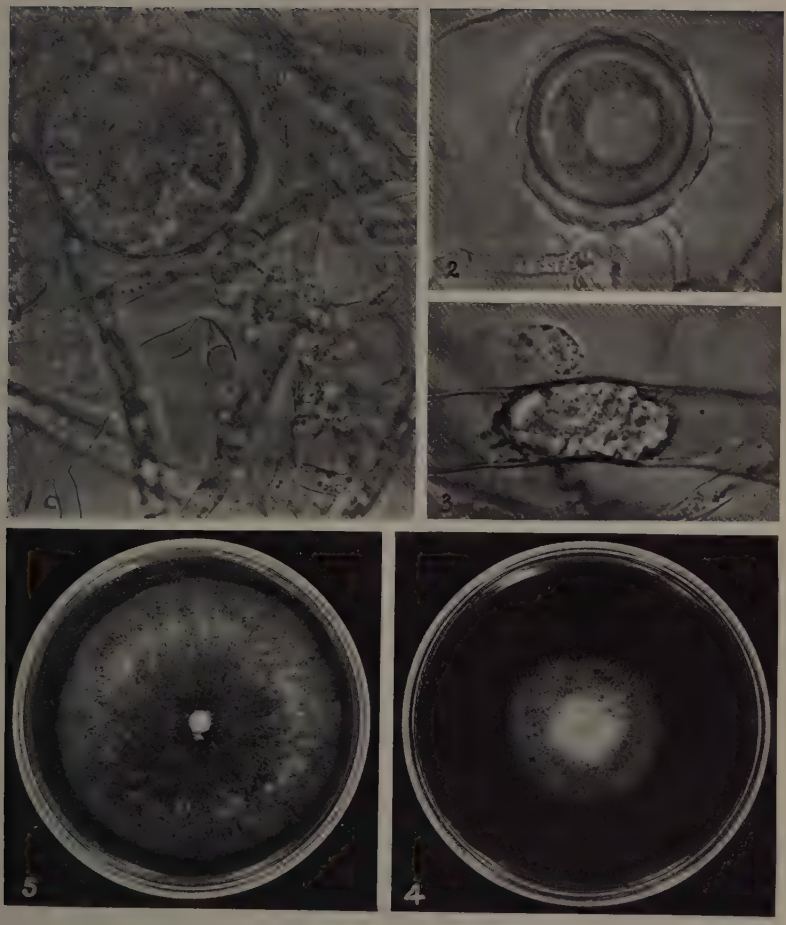
Taxonomic Considerations

The taxonomy of *P. arrhenomanes* var. *canadensis*, including various isolation strains, deserves some discussion. Because of its lobulate sporangia and numerous crook-necked antheridia, it clearly belongs to the subgenus *Nematosporangium* near to the so-called *arrhenomanes* group. If morphological characters alone are considered, a large number of congeneric isolation strains would readily fall into one species, but culturally and to some extent pathogenically, many morphologically similar strains show quite distinct differences. As such divergent differences may occur in a single strain if this is studied for a long time under a variety of conditions, the correct delimitation of species is by no means a simple matter. Other workers have had similar experiences. Edgerton and coworkers (8), after comparing a United States corn *Pythium* and a Hawaiian cane *Pythium* with their Louisiana cane *Pythium* forms, state that:

"While similar in many ways, these cultures have shown some differences . . . . . It is not yet possible to state whether or not the cultures are distinct enough to represent separate species."

Rands (17) also compared the Hawaiian cane *Pythium* with *P. arrhenomanes* and other congeneric forms. He states that:

"Thus, considering all the tests, there are many points of similarity and of difference in cultural behavior when one attempts to compare any two strains. While they are all obviously very closely related, it remains to be seen whether the differences noted may be constantly correlated with morphological characters or parasitic propensities."



FIGS. 1–4 *Pythium volutum.* FIG. 1. *An oogonium with an antheridial branch coiling about the oogonial stalk,* ×900. FIG. 2. *A mature oospore not filling the oogonial sac,* ×900. FIG. 3. *An oblong oogonium in a root hair, showing an antheridium making contact through the cell wall,* ×900. FIG. 4. *A 2-day culture on carrot-cornmeal agar.* FIG. 5. *P. arrhenomanes var. canadensis, a Petri-plate culture prepared and kept under the same conditions as P. volutum in Fig. 4.*

Sideris (21), however, separates *P. arrhenomanes* and other very closely allied forms mainly on a cultural basis, and states in support of his procedure that, "As morphological differences in the shape and size of oögonia, oöspores antheridia, prosporangia, or zoöspores between species of the same section are almost insignificant, rarely exceeding those of normal variation, the adoption of such characters for differentiation would have been misleading. Physiological and certain morphological differences, however, have been found to be fairly constant as well as stable in certain culture media and for this reason they have been adopted for the differentiation and taxonomic classification of the various species."

After conducting morphological, physiological and pathogenical studies on the Wisconsin corn *Pythium*, the Louisiana cane *Pythium*, and the Saskatchewan cultures, the present authors concluded that the form referred to as *P. arrhenomanes* var. *canadensis* in the present paper, should be regarded as a variety of *P. arrhenomanes* because of its close morphological and pathogenical similarities and its cultural differences with this latter species. This was previous to the publication of Sideris' work. After a study of this paper we do not see that *P. arrhenomanes* var. *canadensis* fits exactly any of Sideris' newly erected species, although it is undoubtedly closely allied to the group which reproduces sexually in one to three days on all culture media. Until there is more general agreement among authorities on the group regarding the methods and characters used in delimiting species of *Pythium*, we feel that it would be best to let the name *P. arrhenomanes* var. *canadensis* stand for the present. The decision that it be regarded as a variety of *P. arrhenomanes* is based mainly on the following differences:

1. Its more ready production of sexual bodies in culture.
2. In general, its less ready production of lobulate sporangia in culture.
3. Its slightly larger oogonia and oospores, and,
4. The presence of 3 to 6 antheridia ordinarily applied to each oogonium. Never more than 12 antheridia have been counted, while in *P. arrhenomanes* they are said probably to double this number.

The comparative parasitism experiments with *P. arrhenomanes*, the Louisiana cane *Pythium* and *P. arrhenomanes* var. *canadensis*, as conducted in the greenhouse and in small field-test plots on cereals and corn, revealed no striking differences, but rather confirmed the close relationship of the three parasites as already indicated morphologically.

The morphological and other characters of *P. volutum*, especially the characteristic coiling of the antheridial branches about the oogonial stalk, are sufficiently distinct from those of any other described species of *Pythium* of the lobulate-sporangium group to indicate that it is a new species.

Comparative studies with *P. aphanidermatum*, *P. butleri*, and *P. graminicolum* have shown that both *P. arrhenomanes* var. *canadensis* and *P. volutum* are distinct from any one of these species.

Recent views regarding the much-needed change in the taxonomy of the genus *Pythium* have been put forward by several writers (7, 10, 20, 23), but we

consider it best for the present to refer our forms to *Pythium* in its broader sense. No attempt will be made in this paper to enter into the discussion on the classification of the genus as a whole.

Pathogenicity

Penetration of the roots of the cereal host by the two species of *Pythium* just described occurs readily through both the epidermal and root-hair cells

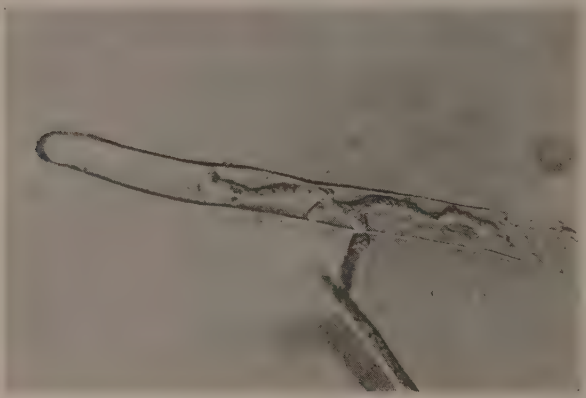


FIG. 4. *A hypha of P. arrhenomanes var. canadensis, penetrating a root-hair cell of a wheat seedling in a water culture. Note the constriction in the hypha at the point of entrance.* ×600.

(Fig. 4). Where the infection hypha comes in contact with the host cell wall, a small appressorium develops and a narrow infection tube pierces the cell wall, and, once inside, regains the normal diameter of the mycelium. The same phenomenon usually occurs when hyphae pass from cell to cell within the host. In so far as can be observed the host offers no resistance to the invading parasite. The growing point of root or rootlet is most commonly attacked, and consequently further growth in length is either retarded or stopped completely. Both fungi grow rapidly through the cortex in all directions and in the course of 24 hr. or less have entered the stele of young roots. Under greenhouse conditions, the affected root tips or girdled portions of the older roots after a time become discolored or necrotic, and present an appearance similar to plants attacked under natural conditions (Fig. 1). It is in these darkened zones that the majority of oospores are found later, although in rare cases of heavy inoculation they may be produced in the coleoptile and the protective sheaths about the crown of the plant. In the pot experiments, oospores do not develop as readily in the discolored root tips as they do under field conditions (Plate I, 1). Browning or yellowing of the outer leaves of seedlings in artificially inoculated soil is occasionally quite characteristic, though ordinarily they remain green even though the root system may be greatly impaired. There is some evidence that sunlight intensity influences these browning symptoms.

It is thus seen that disease symptoms similar to browning root rot in the field can be produced experimentally. Further, re-isolation of the parasites, from the roots of plants in which the disease has been artificially produced, is readily secured.

Comparative Parasitism with other Root-rotting Fungi

An experiment was conducted to ascertain the relative parasitic abilities of *P. arrhenomanes* var. *canadensis* and the well-known foot-rot and root-rot parasites of wheat in this province, namely, *Helminthosporium sativum*, *Ophiobolus graminis*, and *Fusarium culmorum*. The cultures of these last three fungi were the most parasitic Saskatchewan strains obtainable from the Dominion Laboratory of Plant Pathology, Saskatoon, where they have been used in root-rot investigations for some time. All cultures were grown on sterile oat-barley medium in separate flasks and after ten days, 10 gm. of inoculum of each species was placed at seed level in soil contained in 6-in. pots. Twenty grains of wheat were sown to each pot. The soil was kept at a moisture content of approximately 70% of its water-holding capacity. In one series steam-sterilized soil was used and in another it was left unsterilized. The experiment was carried out in duplicate. To one control pot 10 gm. of sterile oat-barley medium was added at seed level.

FIG. 5. *Comparative parasitism of various root-rotting fungi four weeks after inoculation. 1, F. culmorum; 2, H. sativum; 3, O. graminis; 4, P. arrhenomanes var. canadensis; 5, Control, plus oat-barley medium; 6, Control, untreated.*

Fig. 5, taken four weeks after the date of seeding, shows the relative damage of the different parasites in steam-sterilized soil during the seedling stage of development. *H. sativum* and *P. arrhenomanes* var. *canadensis* caused about 75% pre-emergence killing. *O. graminis* did not appreciably affect germination, but post-emergence killing of the seedlings reduced the stand considerably. *F. culmorum* did not reduce germination and retarded growth only slightly.

FIG. 6. *Relative pathogenicity of various species of Pythium on wheat (W), oats (O), and corn (C), four weeks after inoculation. 1, Control plus oat-barley medium; 2, Control, untreated; 3, P. arrhenomanes var. canadensis; 4, P. volutum; 5, Louisiana Pythium 931; 6, Louisiana Pythium 1432; and 7, P. arrhenomanes.*

Little or no disadvantageous effects were produced on the growth of the seedlings by the oat-barley medium. Damage to about the same order of magnitude was caused by the parasites in the unsterilized soil, but the plants in the control pots of unsterilized soil containing the oat-barley medium were often slightly impaired in their development. In another series of experiments in which the inoculum of each parasite was placed one inch below seed level, *P. arrhenomanes* var. *canadensis* showed the most vigorous parasitism up to four weeks.

The results indicated that *P. arrhenomanes* var. *canadensis* may be as vigorous a seedling parasite of wheat as either *H. sativum* or *O. graminis*, and much more parasitic than *F. culmorum*.

Relative Pathogenicity of Species of Pythium on Cereals

Comparative parasitism experiments between *Pythium arrhenomanes* var. *canadensis* and *P. volutum*, and *P. arrhenomanes* Drech., strain 931 and strain 1432 of the Louisiana sugar-cane *Pythium*, *P. graminicolum* Subram., and a few other congeneric forms, were conducted on various hosts in 6-in. pots in the manner already described for the greenhouse experiments. The temperature varied between 60° and 70° F., and the plants were watered daily. Twenty seeds were sown to each pot except in the case of corn when ten were sown.

Fig. 6 illustrates the plants from a single series of pots in a typical experiment conducted in duplicate, while Table I gives the total number of plants in each pot.

TABLE I

COMPARATIVE PATHOGENICITY OF SPECIES OF PYTHIUM ON CEREALS

Host plant	Number of plants in each pot after one month						
	Control + oat-barley medium	Control untreated	P. arrheno-manes var. canadensis	P. volutum	La. Pythium 931	La. Pythium 1432	P. arrheno-manes
Wheat (Marquis)	20	20	11	12	14	13	18
Oats (Banner)	20	20	19	14	19	20	20
Barley (Hannchen)	20	20	12	18	16	13	18
Rye (Prolific)	18	19	6	9	9	4	5
Corn (Squaw)	10	10	5	9	8	4	3

The results of this and other similar experiments show the striking similarity in parasitism between *P. arrhenomanes* var. *canadensis* and Louisiana *Pythium* 1432. Louisiana *Pythium* 931 and *P. arrhenomanes* ordinarily give the same results but these do not compare as uniformly with those of *P. arrhenomanes* var. *canadensis* as do those of Louisiana *Pythium* 1432. They are all severely parasitic on wheat, rye and corn and slightly to moderately parasitic on oats

and barley. *P. volutum* differs in its parasitism from all of these other forms. It is severely parasitic on oats, usually equally as parasitic on wheat and rye, moderately parasitic on barley and only slightly parasitic on corn.

P. arrhenomanes var. *canadensis* reduces the stand and arrests the growth of western rye grass and brome grass, on which it may be classed as moderately parasitic. *P. arrhenomanes* and the Louisiana *Pythium* strains were found to be slightly parasitic on these grasses in a single experiment. *P. arrhenomanes* var. *canadensis* causes a trace of damage on flax and peas in pot trials.

Through the courtesy of Dr. C. Drechsler, the authors were able to obtain a culture of *P. graminicolum* Subram. which he obtained from sugar-cane roots. Subramaniam, whose original culture is no longer extant, found the fungus to be the cause of a crown and root-rot disease of wheat in India. Under our conditions of experimentation it is only slightly parasitic on Marquis wheat compared with *P. arrhenomanes* var. *canadensis* and *P. volutum*. *P. butleri* Subram. was not found to be pathogenic on wheat; neither was *P. aphanidermatum* (Edson) Fitzp. (*P. butleri*?) of the American Type Culture Collection.

The pathogenicity findings substantiate the fact, already arrived at from morphological studies, that *P. arrhenomanes* var. *canadensis* is very closely related to the *Pythium* on corn and the *Pythium* on sugar cane. *P. volutum* is a distinct parasite which is not known to have been described before.

Field Experiments

During 1930 and 1931 various field tests were conducted in small experimental plots artificially inoculated at seed level with oat-barley cultures of the parasites.

In 1930 there were no outstanding differences between the uninoculated rows and rows inoculated with species which proved vigorously parasitic under greenhouse conditions. The inoculated plants did show a few necrotic root-tip lesions, but there was an entire absence of the aggressive parasitism which the greenhouse experiments, in both non-sterile and sterile soil, had led one to expect. The exceptionally dry condition of the soil may have been a factor which rendered the inoculum non-viable before the seed germinated.

The 1931 rod-row results showed definitely that *Pythium* injury to cereals can be obtained from artificial inoculation in the field (Fig. 7). Owing to the dry conditions prevailing, water was applied to the rows at the time of seeding and every two or three days thereafter until the seedlings were about three inches high. Sowings were made on April 30, May 5, May 9, and May 15. The inoculum used was about 7 to 10 days old in each case. The results in the aggregate are in general agreement with those obtained in pot experiments (Fig. 7). Both *P. arrhenomanes* var. *canadensis* and *P. volutum* delayed germination and caused considerable pre-emergence killing. The plants which emerged in the inoculated rows usually grew fairly well, but were always slightly shorter and later than the uninoculated plants. The majority of inoculated plants, however, had varying percentages of necrotic root tips containing *Pythium* oospores. *P. volutum* was more parasitic on both wheat and oats than was *P. arrhenomanes* var. *canadensis*, although they

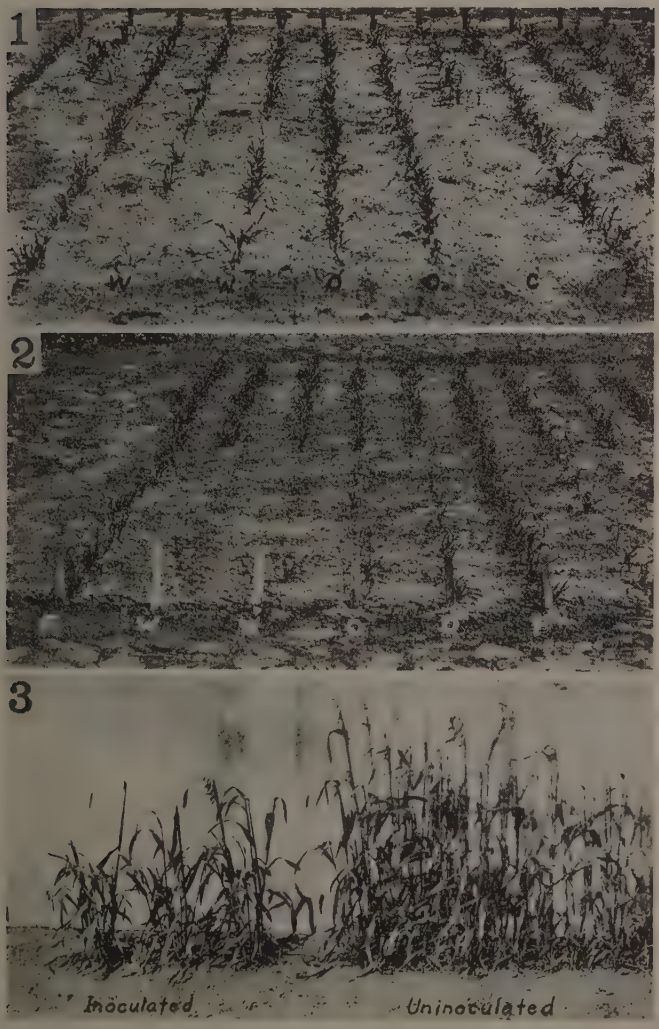


FIG. 7. *The effects of artificial inoculation in the field. In 1 and 2 the front eight feet of each rod row was inoculated, and the remaining eight feet, at the back, was left uninoculated. W, wheat, O, oats; C, corn; and F, filler row of wheat, uninoculated. 1. Inoculated with P. arrhenomanes var. canadensis. 2. Inoculated with P. volutum. 3. P. volutum on oats, showing dwarfing and delayed maturity.*

both caused much more damage to wheat than to oats. Under the field-plot conditions, *P. arrhenomanes*, Louisiana *Pythium* 1432, and the two Saskatchewan forms, are very similar in parasitism on wheat, oats and corn. On the other hand, *P. graminicolum* showed only slight indications of parasitism to wheat under the same conditions. No differences in the effects of various

fertilizers on the disease could be observed in these plot tests.

It was found that the amount of pre-emergence killing was greatly influenced by the state of the inoculum; *i.e.*, whether it had been kept under good growing conditions for 7 to 10 days or whether it had been under adverse conditions or was a little too old. This doubtless interfered with any differences produced by the different dates of seeding.

Too many difficulties attend comparative field experimentation with different species of *Pythium*, and it is felt that a fairly accurate idea of the relative parasitic ability of the various parasites can be obtained from pot experiments in the greenhouse where conditions can be more thoroughly controlled. On the other hand, any clear-cut effects of fertilizers on the disease will probably be ascertained only by conducting experiments in naturally infested fields.

Soil Temperature and Moisture Relationships

To ascertain the influence of soil temperature and moisture on the infection of young wheat plants by *P. arrhenomanes* var. *canadensis*, experiments were conducted in galvanized iron soil cans, 6 in. in diameter and 10 in. deep, in temperature-controlled tanks.

The soil was made up to the required moisture content by weight based on its water-holding capacity. A layer of sterile sand $\frac{1}{4}$-in. thick was placed on top to prevent the soil from caking or cracking. Surface watering was found to give as good a distribution of moisture as any other method of application. Weighings were made every other day and the necessary amount of water added to maintain the correct moisture percentages. Four temperature tanks containing eight cans each were used. These were maintained at 12°, 17°, 24° and 31° C. respectively, and the air temperature at approximately 17° C.

Inoculations and sowings were carried out in the manner already described for greenhouse pot experiments.

Moisture relations.—From the preliminary temperature-moisture experiments it was clearly shown that the amount of damage to wheat seedlings at all the temperatures increased with increasing moisture content of the inoculated soil.

As a result of this finding, all subsequent temperature-controlled experiments were conducted with a soil moisture content of about 70%.

Temperature relations.—Fig. 8, A, shows plants from a single representative temperature-tank experiment after four weeks. Each bundle represents the final stand from four cans.

The results of this and other temperature-tank experiments indicate that:

(1) The amount of damage to wheat seedlings in inoculated soil, *relative to the controls*, increases directly with increase in temperature. The disease rating was estimated on the percentage of seedlings in the final stand, their oven-dried weight, their average height, and the conditions of their root systems. Actual damage in the inoculated cans is least at 24° C. and increases towards the two lower temperatures as well as towards the higher. It is however consistently worse at 31° C. than at 12° C.

FIG. 8. *The relation of temperature to the disease. A, each bundle represents the final stand after four weeks from eighty seeds inoculated at time of sowing in a single temperature-tank experiment. For each temperature (as indicated in the figure) the bundle on the left represents the control plants and that on the right the inoculated plants. B, for each temperature as indicated, the bundle on the left shows the inoculated plants and those on the right the uninoculated. Inoculation was performed after the seedlings had emerged; both inoculated and controls were then kept at the respective temperatures for two weeks.*

(2) Pre-emergence killing is greatest at the lowest and highest temperatures, although usually slightly worse at the highest.

(3) Post-emergence killing is worst at the highest temperature. The final stand is therefore least at the highest temperature.

Under field conditions the disease symptoms first appear when the seedlings are from 4 to 6 in. high, and, so far as is known, neither pre-emergence killing nor post-emergence killing is common. To circumvent the damage caused in the germination stages in the greenhouse experiments, it was thought necessary to devise some method of inoculating the wheat seedlings after they had reached a height of 1 to 3 in. The following procedure was found well adapted to the particular case in question, and, it seems, might well lend itself to experimentation with other root rots of cereals and other plants. Five disinfected wheat grains were sown in sterilized soil in 2-in. pots, and when the seedlings were 1 to 2 in. high, fifteen seedlings, *i.e.*, the contents of three small pots, were transplanted to a single 6-in. can, care being taken not to damage the root systems or disturb the soil unnecessarily. The cans contained sterilized soil in which 20 gm. of inoculum had been incorporated. Each of the three individual batches of seedlings was so placed that inoculated soil came in contact with the roots on all sides. In the control cans no inoculum was added to the sterilized soil. The cans were then placed in their respective temperature tanks and the soil kept at a moisture content of about 70%.

Fig. 8, B, shows a representative series in an experiment conducted in the manner just described. The seedlings were transplanted on the seventh day and then kept at the respective controlled temperatures for two weeks. The oven-dried weights of these plants showed that the damage at 12° C. was approximately 49%, at 17° C. 51%, at 24° C. 60%, and at 31° C. 76% of the control plants at the respective temperatures.

It seems that *P. arrhenomanes* var. *canadensis* can cause severe damage to young wheat seedlings when inoculation is performed after the plants have emerged, that the amount of damage relative to the controls increases directly with increase in temperature, and that post-emergence killing or seedling blight can occur. These results are in agreement with those obtained when inoculation is done at the same time as sowing.

Hydrogen Ion Relationships

On the disease. -A preliminary study on the relation of the hydrogen ion concentration of the soil on the disease was made by testing the pH of browning root-rot soil and of soil from healthy areas in the same field, and from healthy summerfallow wheat fields during the early growing season. Both the infested and the healthy soils tested were either neutral or slightly alkaline, so that although these conditions may be favorable to the root rot, soil reaction need not be considered of any consequence in the disease situation in the province. Attempts were made in the greenhouse to grow wheat in inoculated soil adjusted to pH values ranging from 3.5 to 9.0. Sulphuric and hydrochloric acids and sodium and potassium hydroxides were used in the soil-reaction adjustments. It was found practically impossible with the prairie soils to maintain an equilibrium at a constant pH on the acid side of neutrality for a sufficiently long time to conduct a worthwhile experiment. This is in all

probability due to the large reserve of carbonates in the prairie soils. In the tests that were made, there were no definite indications to show that acid soils decreased the disease to any appreciable extent.

On the parasites.—Cultural studies have shown that *P. arrhenomanes* var. *canadensis* and *P. volutum* will grow in nutrient solutions with a much lower pH value than that of any prairie soil tested, but that the optimum growth for both species occurs at neutrality. Comparative pH studies have shown the very close affinity between *P. arrhenomanes* var. *canadensis* and the Louisiana *Pythium* 1432 (11) and also suggest that this physiological study may be useful in helping to ascertain the identity of those *Pythium* forms which fail to produce fruit bodies in culture.

A detailed study of the hydrogen ion relationships of *P. arrhenomanes* var. *canadensis* and *P. volutum* will be published in a separate communication.

Fertilizer Experiments

Several fertilizer treatments were conducted under greenhouse conditions on wheat grown in a mixture of browning root-rot soil collected from four localities, in sterile soil inoculated with an oat-barley culture of the parasite, and in field-test plots artificially inoculated. The effects of the following fertilizers were tried, namely, sodium nitrate, ammonium sulphate, potassium sulphate, potassium chloride, ammonium phosphate, triple superphosphate, straw and farmyard manure; especial attention was given to the effects of nitrogenous fertilizers on the disease.

The results failed to show any appreciable differences on the amount of root rot from the various treatments. However, the following general observations may be noted:

1. In the majority of instances, no noteworthy differences in the amount of root lesioning occurred with the various fertilizer amendments. The fertilizer treatments produced various effects on the host, such as differences on growth rate, rather than on the disease.

2. The plants treated with sodium nitrate in a few cases produced discolored coleoptiles and a darker root system than the controls. Usually, however, there were no differences in roots or tops between the nitrate-treated plants and the controls, except that the nitrate-treated plants were greener. Normally, one would have expected an increased growth in tops and roots over the controls.

3. Straw either produced no difference or the plants were better than the controls throughout. This is also contrary to normal expectations.

4. Farmyard manure gave much the same results as straw.

Carpenter (2) contends that *Pythium* root rot of sugar cane in Hawaii is enhanced by nitrogenous decomposition compounds of sugar-cane factory by-products and by excess nitrate; also that bagasse or pure sugar-cane fibre inhibits the disease. Our greenhouse experiments coupled with the knowledge that the nitrate nitrogen is highest when browning root rot is worst, and that on the stubble crop nitrate nitrogen is low when browning root rot is practically absent, suggest that Carpenter's view may hold for browning root rot of wheat

also. This is only suggestive as present results are indefinite and inconclusive; it remains for fertilizer experiments in naturally infested fields to prove or disprove this view.

Seed Treatments

Since the parasitic species of *Pythium* are extensively distributed in the soils of the province and are not seed borne, the commonly practised seed treatments are of no significance in preventing damage from root rot. On the contrary, it has been shown experimentally in the greenhouse that those seed treatments which tend to delay or slightly decrease germination, indirectly increase the amount of a pre-emergence and early seedling damage, possibly because of a slightly weakened condition and because the seedling is exposed to attack from the parasites for a longer time before it can become well established. Greenhouse experiments in inoculated soil indicate that deep seeding with its consequent delayed emergence also reduces germination and increases early seedling damage.

Discussion

It is relatively easy to demonstrate the pathogenicity of species of *Pythium* isolated from the diseased roots of cereal seedlings, but the elucidation of the predisposing factors which operate under field conditions presents more difficulty. The relationship of environmental factors to browning root rot is most important. The amount and intensity of sunshine in the field is believed to have a definite bearing on the expression of disease symptoms, but these factors may be operating only in a limited way when inoculation experiments are conducted in the greenhouse. Thus, in our experiments, root lesioning and injury was usually typical, while the parts above the ground rarely showed the brown discoloration of the outer leaves. The parasitic fungi have been shown to be present in the majority of our wheat-growing soils, so that the immediate problem centres around the edaphic and climatic factors which make the seedling roots, especially on summerfallowed land, susceptible to fungous invasion. It is possible that unbalanced nutrition at this time may play a contributing part. A lag in the growth of the seedlings is brought about in soil of otherwise excellent tilth and with plenty of available moisture. These soil conditions probably favor the rapid spread of the parasitic fungi. The disease is most common in wheat for the simple reason that wheat is the crop almost invariably grown on summerfallowed land.

It is a known fact that under prairie crop culture conditions, the nitrate-nitrogen content of the soil is greatest in May and June in the wheat crop following summerfallow (15, 27, 28), and it is possible that the high nitrogen content of the soil at this time may render the wheat roots susceptible to attack. This contention is supported by evidence in current literature which shows that nitrogenous fertilizers render numerous host plants more susceptible to fungous attack, and especially by the finding of Carpenter (2) that *Pythium* root rot of cane is increased by the application of excess nitrogenous amendments. From numerous fertilizer experiments conducted in pots and in artificially inoculated field plots we have so far been unable to detect any consistent out-

standing effect of nitrogenous fertilizers on the incidence of this disease. The indication is that the direct effect of various fertilizers on the growth of the plants appears to be of greater importance than their effect on the severity of browning root rot. However, the results are in no way conclusive and further fertilizer experiments conducted in naturally infested fields may yield very interesting results.

All evidence indicates that the disease is favored by high soil moisture conditions; also that its severity increases with increase in temperature, but that aggressive parasitism occurs over a wide temperature range providing other conditions are favorable. On the other hand, *Pythium* root rot of corn (12) and of cane, both caused by forms identical or very closely allied to the wheat *Pythium*, are most severe at relatively low temperatures. It appears that here is a case where the optimum temperature for the disease is determined by the particular host plant which the fungus attacks. Wheat is more likely to be adversely affected by higher temperatures and therefore becomes more susceptible at these temperatures while with corn and cane the converse is true. However, the incidence of the disease on wheat and corn under controlled temperatures should be conducted under identical conditions. Such an experiment is contemplated. What appears to the authors to be an analogous instance has already been reported by Dickson (4) for *Gibberella saubinetii* (Mont.) Sacc. attacking corn and wheat.

Under natural field conditions *Pythium* injury rarely manifests itself in reduced germination or as post-emergence dying of the seedlings, but nearly always as a root rot which reduces the size and vigor of the growing plants. That these plants may frequently show marked recovery and mature normally, albeit somewhat later than healthy plants, indicates the lack of production of any toxic substance by the fungous parasite. However, in such cases the injury is expressed in a reduction in yield of three to ten bushels per acre. In pot experiments reduced germination and post-emergence blighting are more common.

Nematodes were found on wheat in one browning root-rot locality, but it is believed that they are of no significance in the disease situation.

A discussion of the taxonomic relations of *P. arrhenomanes* var. *canadensis* and *P. volutum* has already been dealt with earlier in this paper, and will not be considered here.

Finally, it should be pointed out that, from a pathogenic standpoint, the sphero-sporangium forms may be regarded as of little consequence, while the nematosporangium forms, which have been given most attention in these investigations, are of major importance.

Acknowledgments

The authors are grateful to Drs. Helen Johann, C. Drechsler, C. W. Carpenter, E. C. Tims, and G. A. Ledingham for their courtesy in supplying various *Pythium* cultures for comparative studies.

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THE SOLUBILITY OF HYDROGEN SULPHIDE IN WATER FROM THE VAPOR PRESSURES OF THE SOLUTIONS[1]

By R. H. Wright[2] and O. Maass[3]

Abstract

The vapor pressures of a number of solutions of H_2S in water have been measured at temperatures between 5° and 60°C. A new type of glass diaphragm manometer having several advantages is described, and a bibliography of flexible glass manometers is given. The results show that Henry's law is not strictly obeyed and that previously reported values may require correction. Discussion of the results is reserved for a later paper.

Introduction

Aqueous solutions of hydrogen sulphide are so widely used for such a variety of purposes that a close examination of their properties is important. In this paper is described a series of measurements designed to give the solubility of hydrogen sulphide in water at a number of temperatures and at pressures other than atmospheric; and leading also to a determination of the degree to which Henry's law is applicable. In a subsequent paper will be given the results of electrical conductivity measurements with similar solutions, determinations of the extent of primary dissociation, and an attempt to explain the results of both papers in terms of kinetic equilibria between molecular species existing in the solution.

Previously recorded data on the solubility of H_2S in water suffer from the incorporation of Henry's law as an implicit assumption, a part of the experimental method. Since the authors have found that the law is not strictly obeyed (although the deviations are not large) the last significant figures of the accepted values (4, 11, 12 p. 259, 18, 21, 22) are probably invalidated at atmospheric pressure, and most certainly so at all other pressures.

The present measurements are based on determinations of the equilibrium pressures of known mixtures of hydrogen sulphide and water confined in an especially designed cell. The results given by this method are more directly applicable than those obtained by the usual gravimetric and volumetric procedures.

The experimental arrangements here described were developed for the purpose in hand, but there are features that may prove useful in other work.

Purification of Materials

The hydrogen sulphide was obtained from a cylinder of the liquefied gas and purified by a process of fractional distillation that has been elsewhere described (23). Laboratory distilled water was used and freed from dissolved gases by repeated freezing and melting *in vacuo*.

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada. Constructed from a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry at McGill University.

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Measurement of Vapor Pressures in a Closed System: The Glass Diaphragm Manometer

Using H_2S, it was desirable to employ an all-glass vapor pressure cell to restrict the possibility of stray reactions between the gas and mercury or stopcock grease. None of the hitherto described devices* depending on the flexibility of glass for the indirect measurement of pressure in a closed system appeared to be suitable for this work, and eventually a new variety was evolved combining many of the best features of the previous types (*e.g.*, visibility of the pointer, indifference to temperature, etc.). The instrument is, moreover, fairly easily made.

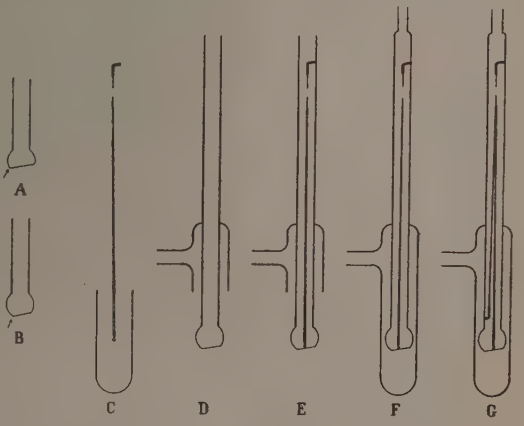

FIG. 1. *Stages in building glass manometer.*

The various steps in making the device are shown in Fig. 1, and its application to the measurement of vapor pressures in Fig. 3. The sensitive diaphragm was blown at the end of a long tube, ring-sealed into a larger tube closed at the bottom and provided with a side tube for connection to the vapor pressure cell. Affixed to the inside of the diaphragm was a light, thin, glass pointer, long enough to project well above all parts of the cell containing the gas (thus allowing for complete immersion in the thermostat). Vibration of the pointer was damped by filling the tube with light oil. It must be emphasized that to produce a satisfactory diaphragm the form of flattening shown at *A*, Fig. 1, must be avoided, since the relatively acute angle between bulb and flattened portion results in a very easily broken diaphragm. By making the flattened part smaller, as shown at *B*, a far thinner yet stronger diaphragm may be made.

At *G*, Fig. 1, is shown an alternative type of pointer giving a somewhat greater sensitiveness.

In order to magnify the movements of the pointer, the magic lantern principle was used. By a suitable lens system, enlarged images of the (blackened) tips of the pointer and index (shown in the diagram) were projected on a screen some distance away. Prior to commencing a run, the null position of the pointer with respect to the index (with equal pressures on opposite sides of the diaphragm) was noted. In order to measure unknown pressures thereafter, the external pressure needed only to be made approximately equal to the unknown, for, within a considerable range, the deviation of the pointer from the null point was proportional to the pressure difference producing it, and so,

**A bibliography of flexible glass manometers is given at the end of this paper.*

once the proportionality constant had been found, the internal pressure was quickly obtained. This feature, together with the mechanical advantages of the instrument* and the wide visibility of the pointer image, make the instrument of very great value. In this work, a precision of 0.2% was sufficient, and an instrument reading to 0.5 mm. was easily made. Greater sensitivity could have been obtained if necessary.

Method of Charging the Vapor Pressure Cell

Before determining the vapor pressures of the solutions it was necessary to admit known, and within limits, predetermined amounts of hydrogen

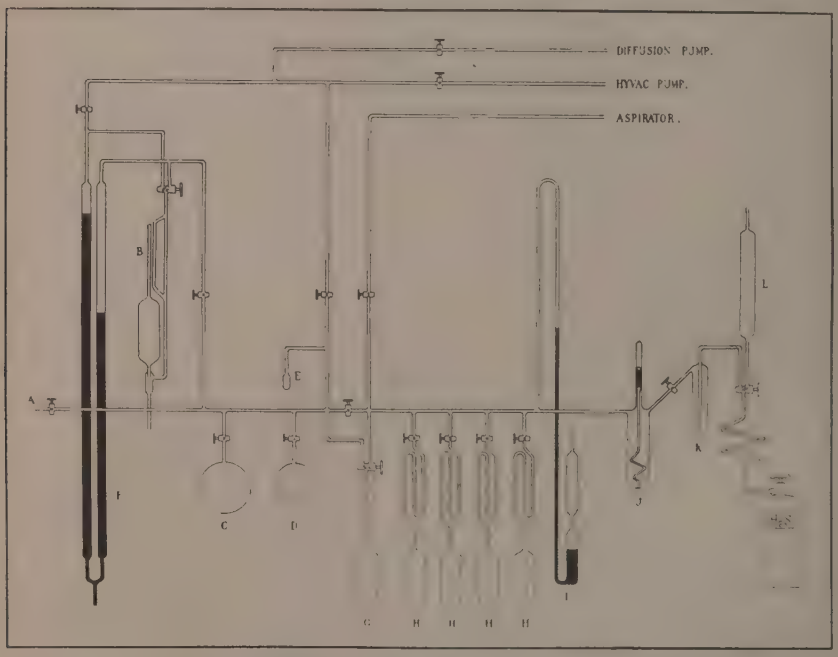

FIG. 2. *Gas measuring apparatus.*

sulphide, into the cell. This was done by the following method. The gas was admitted to a bulb of known volume at a known temperature and pressure and its weight calculated from the vapor density. The tubing was then evacuated and the measured amount of gas condensed in the cell with liquid air. The measuring apparatus is shown in Fig. 2, *G* being the storage bulb for purified hydrogen sulphide, *C* and *D* the calibrated volumes (thermostated at 0° C.), and *F* a manometer reading to 0.1 mm. The vapor densities were determined by us and have been published (23). The estimated precision of the method is 0.1%.

**Note that accidental movements of the lens system or scale, or even of the instrument itself, can be remedied without it becoming necessary to re-determine the null point, a most conspicuous advantage over any other type depending on optical magnification.*

The vapor pressure cell, M, is shown in Fig. 3. It contained an electromagnetic stirrer and there was an additional side arm not shown in the figure through which the water was introduced. The U-tube, N, was provided to trap any water that might be removed during the extraction of dissolved air as described below. The apparatus of Figs. 2 and 3 was connected at A.

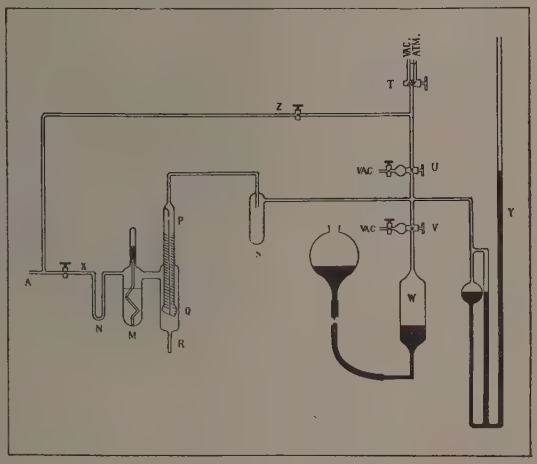

FIG. 3. *Vapor pressure cell.*

Before commencing a run, the cell and attached manometer were removed from the apparatus and the volume found by weighing when filled with distilled water. They were then cleaned and dried and resealed to the apparatus. A known amount of water was run into the cell, M, from a weight pipette and the side arm sealed. Dissolved air was thoroughly removed by repeated freezing and melting of the water *in vacuo*.

With the purified water frozen in M, a suitable amount of hydrogen sulphide was measured out as described and condensed in R with liquid air. The system was sealed off at X and allowed to come up to room temperature. Thus, after the commencement of a run the gas came in contact with nothing but Pyrex glass. The cell was arranged so that a water bath could be brought round it. The temperature of the bath was varied between 5° and 60° C. as desired, and hand regulated to 0.1° C. One or two hours were generally

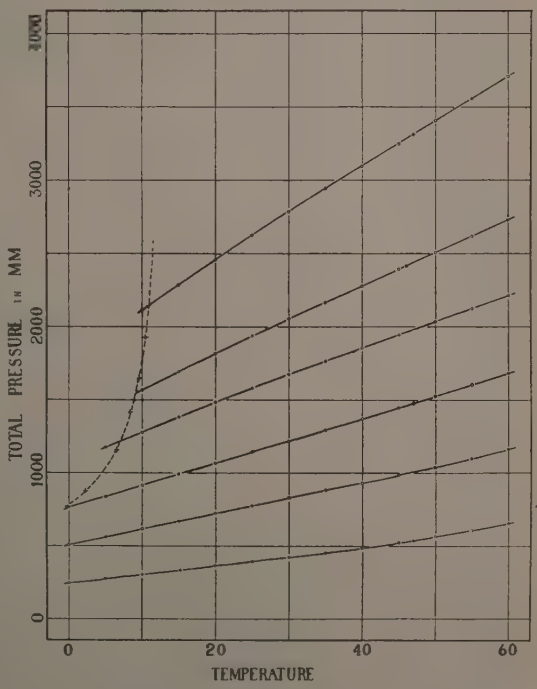

FIG. 4. *Vapor pressure-temperature relation of hydrogen sulphide solutions.*

allowed for the mixture in the cell to come to equilibrium after each temperature change. The method of measuring the vapor pressure has been described.

Experimental Results

The observed pressures were reduced to mm. Hg. at 0° C., and plotted against temperature as in Fig. 4.* (The black circles represent experimental, and the open circles interpolated, values.) Each curve in Fig. 4 represents one run. From the total pressures as observed or interpolated, the internal volume of the cell and the amounts of material in it, the concentration of the solution was calculated at each temperature with the help of the equation:

$$P_1 - P_2 - \frac{WR}{MV} \cdot T + \frac{wR}{MV} \cdot T = 0,$$

where P_1 is the observed total pressure; P_2, the vapor pressure of water at temperature $T°K$; W, the total weight of hydrogen sulphide in the cell; w, the weight of dissolved hydrogen sulphide in the solution; R, the gas constant in cc.-mm.; M, the apparent molecular weight of hydrogen sulphide at temperature T and pressure P_1-P_2; and V, the volume of the vapor phase in cc.

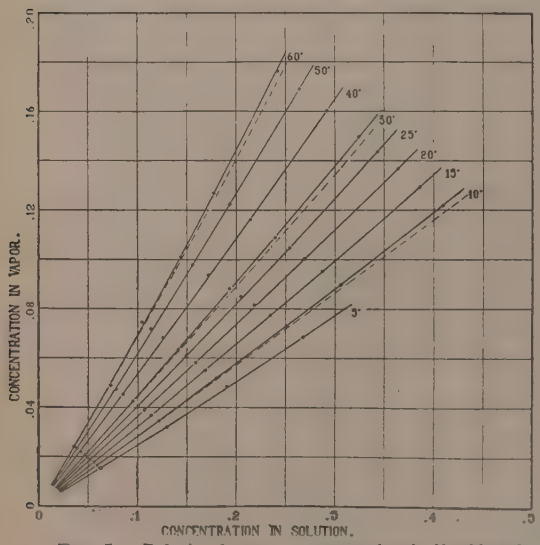

FIG. 5. *Relation between concentration in liquid and concentration in vapor.*

These terms were subject to various corrections. P_1 and P_2 were in mm. of Hg. at 0° C. The apparent molecular weight, M, was calculated from the data of the preceding paper (23).

The volume of the vapor phase, V, was found as follows: assuming the total volume of the cell to be constant over the temperature range covered, the volume of the water was calculated at each temperature and then corrected (by the mixture rule) for the amount of dissolved hydrogen sulphide as obtained from an approximate calculation. The theoretical molecular weights of water and hydrogen sulphide were taken as 18.02 and 34.08 respectively. The equation was solved

**Separation of "H_2S hydrate" from the more concentrated solutions at low temperatures prevented the determination of 5°C. equilibrium points on the higher curves. The position of the phase equilibrium line as determined by de Forcrand (7, 8, 9) is rather indefinite. The dotted line in Fig 4 is plotted from points determined by us:—*

Temp., °C.	*2.2*	*6.6*	*8.4*	*9.7*	*10.5*
Moles H_2S per litre	*0.211*	*0.259*	*0.297*	*0.333*	*0.378*
Total pressure, mm.	*816*	*1190*	*1460*	*1700*	*1960*

for w, the weight of dissolved hydrogen sulphide, and the concentration of the solution then calculated in any desired units.

Table I summarizes the results for each temperature, a cross section of all the results being shown for each temperature. The tables show the total pressure, the partial pressure of hydrogen sulphide (in mm.), concentration of hydrogen sulphide in the vapor and liquid phases (gram-moles per litre),

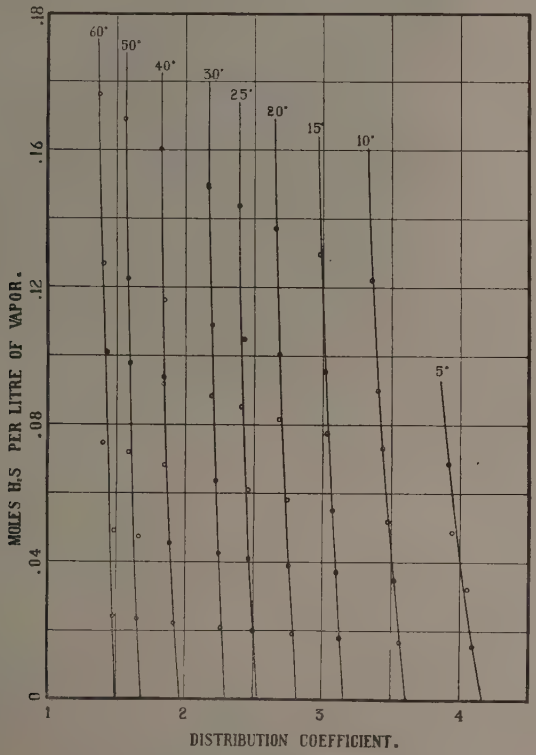

FIG. 6. *Relation between partition coefficient and concentration in the vapor.*

and the partition coefficient, D, of hydrogen sulphide between the two phases. This last has been used instead of the conventional Henry's law constant since it makes due allowance for non-ideality of the gas phase and any variations in it are therefore governed solely by the state of the liquid. For this reason also, in Figs. 5 and 6, concentrations in the liquid and partition coefficients are plotted against the concentration of hydrogen sulphide in the vapor instead of the partial pressure.

Table II summarizes the results and compares them with the values in the International Critical Tables (12). (Table II gives mole fractions divided by partial pressures in mm.) The deviation from Henry's law is evident from the tables and also Fig. 6.

The discussion of these measurements will be given in a subsequent paper.

TABLE I

EXPERIMENTAL RESULTS

Total pressure, in mm.	Partial pressure, in mm.	Moles H_2S per litre of vapor	Moles H_2S per litre of solution	Partition coefficient D	Total pressure, in mm.	Partial pressure, in mm.	Moles H_2S per litre of vapor	Moles H_2S per litre of solution	Partition coefficient D
At 5°C.									
274.5	268.0	0.0155	0.0635	4.09	838	831	0.0484	0.1910	3.94
560	553	0.0321	0.1302	4.06	1176	1169	0.0685	0.2682	3.92
At 10°C.									
303.8	294.7	0.0168	0.0597	3.56	1279	1270	0.0731	0.2511	3.44
615	606	0.0346	0.1220	3.52	1567	1558	0.0900	0.3060	3.40
914	905	0.0518	0.1801	3.47	2112	2103	0.1221	0.4099	3.36
At 15°C.									
333.3	320.6	0.0179	0.0560	3.13	1382	1369	0.0774	0.2346	3.03
670	657	0.0369	0.1144	3.10	1692	1679	0.0953	0.2877	3.02
991	978	0.0551	0.1693	3.08	2284	2271	0.1297	0.3866	2.98
At 20°C.									
362.8	345.4	0.0190	0.0528	2.78	1483	1466	0.0816	0.2188	2.68
724	707	0.0390	0.1074	2.76	1817	1800	0.1005	0.2696	2.68
1067	1050	0.0581	0.1594	2.74	2454	2437	0.1371	0.3642	2.66
At 25°C.									
392.6	369.1	0.0199	0.0497	2.50	1581	1557	0.0851	0.2050	2.41
778	754	0.0409	0.1010	2.47	1935	1911	0.1049	0.2544	2.42
1144	1120	0.0610	0.1499	2.46	2622	2598	0.1437	0.3437	2.39
At 30°C									
422.8	391.3	0.0208	0.0470	2.26	1672	1640	0.0882	0.1932	2.19
830	798	0.0425	0.0955	2.25	2052	2020	0.1091	0.2398	2.20
1219	1187	0.0636	0.1413	2.22	2785	2753	0.1498	0.3247	2.17
At 40°C.									
486.5	431.6	0.0222	0.0426	1.92	1853	1798	0.0937	0.1722	1.84
934	879	0.0454	0.0858	1.89	2278	2223	0.1162	0.2149	1.85
1370	1315	0.0682	0.1260	1.85	3095	3040	0.1603	0.2921	1.82
At 50°C.									
562.4	470.4	0.0235	0.0387	1.65	2033	1941	0.0979	0.1560	1.59
1040	948	0.0474	0.0789	1.66	2505	2413	0.1223	0.1937	1.58
1522	1430	0.0719	0.1139	1.58	3402	3310	0.1690	0.2647	1.57
At 60°C.									
652.2	503.3	0.0243	0.0359	1.48	2213	2064	0.1010	0.1440	1.42
1162	1013	0.0492	0.0730	1.48	2731	2582	0.1269	0.1777	1.40
1681	1532	0.0747	0.1045	1.40	3707	3558	0.1762	0.2424	1.38

TABLE II

SUMMARY AND COMPARISON OF RESULTS WITH THOSE IN THE INTERNATIONAL CRITICAL TABLES

Temp., °C.	Mole fraction / Partial pressure ×10⁶				Temp., °C.	Mole fraction / Partial pressure ×10⁶			
	I.C.T. value	1 Atm.	2 Atm.	3 Atm.		I.C.T. value	1 Atm.	2 Atm.	3 Atm.
5	4.185	4.22	4.12	4.04	30	2.161	2.18	2.17	2.16
10	3.594	3.62	3.57	3.54	40	1.767	1.79	1.78	1.77
15	3.111	3.14	3.11	3.10	50	1.488	1.52	1.50	1.49
20	2.727	2.75	2.74	2.72	60	1.279	1.34	1.31	1.30
25	2.416	2.43	2.42	2.41					

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DISPERSION AND SELECTIVE ABSORPTION IN THE PROPAGATION OF ULTRASOUND IN LIQUIDS CONTAINED IN TUBES[1]

Part I

By R. W. Boyle[2], D. K. Froman[3] and G. S. Field[4]

Abstract

An experimental study by the ultrasonic method of the phase velocity of longitudinal waves transmitted in liquids contained in tubes. Greatly augmented as well as largely decreased velocities may be obtained in any liquid by suitably adjusting the frequency of the wave or the diameter of the containing tube. This phenomenon, described here at length for the first time, is found to be caused by the selective absorption of energy of the longitudinal wave at certain frequencies, resulting in a velocity-frequency curve analogous to the "anomalous dispersion" curve of optics. In the experiments there is strong indication that the absorbing frequency depends inversely on the diameter of the tube.

The fact that the absorption frequency does not depend on the material or length of the tube, or for thin walls on the wall thickness, indicates that it is neither longitudinal nor flexural (lateral) vibrations in the tube walls which causes the phenomenon; and the fact that for any liquid the critical frequency shifts with change of diameter indicates that it is in the column of liquid itself that the energy absorption or transference takes place.

These experiments show that it is only at frequencies far removed from absorption, *i.e.*, on the regular and flat portions of the velocity-frequency curve some distance from the discontinuity, that the usual theories of sound transmission may safely be applied.

Introduction

The modern uses of short-length ultrasonic waves have introduced new and convenient methods by which the velocity of sound in solids, liquids and gases may be more completely investigated. New and interesting results by this method on the velocities in liquids contained in tubes prove that it is easily possible to cause at will largely augmented as well as diminished phase velocities; that while the thickness of the tube wall, its elasticity and density may have a certain small importance, these factors are not nearly as effective in causing marked changes in phase propagation in liquids as the frequency of vibration and the diameter of the vibrating liquid column. While lateral waste of energy in the tubular walls and viscous damping in the liquid may be influencing factors on the phase velocity in the liquid column, the dominating factors fixing the velocity are the factors of motional impedance and selective absorption. The experiments here described offer abundant proof of this conclusion, and there are theoretical reasons as well which are discussed in Part II of this paper.

The Helmholtz-Kirckhoff theory was propounded to explain the diminution of the velocity in fluids contained in tubes, which was almost invariably observed by previous experimenters. While some researchers have claimed that this theory sufficiently explained the facts, others were of the opinion that no theory adequately accounted for them all. Most of the experimental

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work in tubes has been performed on gases, generally air, and in this connection Cornish and Eastman (6), whose work lends support to the Helmholtz-Kirckhoff theory, recently summarized much of the previous research on this important question.

Relatively little experimental work has been carried out in liquids as the contained fluid; but it may be recalled that Dörsing reported certain cases of increase of velocity as compared with the velocity in the same liquid when unconfined. Dörsing's (7) method of measurement was that of the Kundt's tube, and, unlike most of the previous experimenters in this subject, he worked at rather high, though audible, frequencies, about 4000 vibrations per sec. These notes were generated by friction, by rubbing metal rods longitudinally with a motor driven friction device. The conclusions Dörsing reported relevant to the experiments of this paper are summarized as follows: "(1) The velocity of sound in liquids contained in tubes, contrary to the case for gases, *increases* with decreasing radius for a given strength of wall, and for a given radius *increases* for increasing wall strength. (2) When the velocity *decreases* in tubes, the decrease may be ascribed almost exclusively to the elastic forces of the tube and of the liquid; heat conduction and friction are without appreciable influence. (3) Vibrating liquid columns generate sympathetic longitudinal vibrations in the tube wall; hence to obtain Kundt's dust figures, it is desirable to make the natural frequencies of the liquid and tube as nearly equal as possible. (4) In vibrating liquids, contrary to the behavior of gases, the harmonic overtones distinctly assert themselves." In these conclusions are found certain important considerations not comprised in the Helmholtz-Kirckhoff or any similar theory. Busse (5) reverted to Dörsing's method to determine velocities of sound in many liquids for the purpose of determining certain useful thermodynamical constants.

On theoretical considerations there should be corrections applied to the unconfined velocity in a fluid for the waste of energy laterally in the vibrations of the wall. If the wall is not rigid, a damping of the vibrations in the contained fluid is caused by the wall vibration, which damping causes a diminution of phase velocity in the contained fluid. This diminution, however, is usually small.

This phenomenon was predicted by Helmholtz in 1846 and first observed by Wertheim in 1847. Since then the problem has been investigated mathematically by Lamb (11, 12), Green (8), and others, and most recently by Gronwall (9). The last undertakes an exact solution of the problem to find the relation between the velocity of sound C in the column of liquid in the pipe or tube and the velocity C_0 in an unlimited body of the liquid. As might be expected the relation is extremely complicated, but by employing suitable approximations the equation may be reduced to $C_0 = \frac{C}{1-E}$, where $C = 2Ln$, L being the length of a liquid column under fundamental resonance, and n the resonant frequency of the generating note; E is a complicated function of the inner and outer radii of the tube wall, the chief elastic constants of its material, and the density of the liquid. Pooler (13) found verification of Gronwall's relation

experimentally by determining the velocities in a column of liquid contained in a vertical cylindrical steel tube. The column was brought into resonance *at an audio frequency* by an electromagnetically excited diaphragm at the bottom. When the resonance frequency of the liquid column was the same as that of the diaphragm the reaction of the diaphragm on the system was very small, and the velocity under this condition was easily measured.

Virtually all velocity of sound measurements in liquids contained in tubes, with those of Dörsing, Busse, and Hubbard and Loomis (10) as notable exceptions, have been made at low, *i.e.*, audio frequencies, and consequently in tubes whose diameters were very small compared with the length of the wave. It is this fact more than any other which has heretofore masked the effect of selective absorption which by the use of ultrasonics may be easily disclosed. Some of the results of the present paper support Dörsing's observations, but they offer a different explanation for a few of his observed facts.

General Method

The object of the researches here described was to study by the ultrasonic method the phase velocities in cylindrical tubes when the propagating medium is a liquid. It was first intended to use tubes of material in which the specific acoustic resistance (ρV), where ρ is the density and V the velocity of sound in the material, was less than, or comparable with, that of the liquid. In such tubes, the walls could not be considered "rigid" in the theoretical sense. But in the progress of the work it soon became evident that this consideration in comparison with others later described was not of great importance, and it became more convenient to use tubes more nearly "rigid" than at first intended. The frequencies employed in the experiments ranged from about 10,000 to 200,000 cycles per sec., and the phase velocities were measured by the method of stationary waves produced by reflection from a reflector in the tube, or, as in later experiments, by identical oscillators facing one another from opposite ends of the tube.

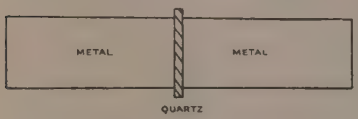

FIG. 1. *Metal rod oscillator.*

The source of ultrasound in the experiments was a simple metal rod oscillator (Fig. 1), or two oscillators, set into longitudinal vibration by the piezoelectric action of a thin plate of quartz appropriately cut. The simple types of oscillators as used in these experiments have often been described, as well as their method of operation by a high frequency electrical oscillation circuit. The metal rods used in the oscillators were generally duralumin, with diameters slightly less than the internal diameters of the experimental tubes. Their lengths were sufficient to permit the convenience of employing the same oscillator without change of experimental arrangement at the first few overtone frequencies as well as the fundamental. The extreme end of the oscillator was fitted snugly inside the experimental tube at one end, the rest of the oscillator being free from the tube but supported at its middle in a thin wooden stirrup. Leaking of the experimental liquid between the oscillator and wall of the tube was prevented by a light packing of soft rubber or suitable

wax. The earliest experiments, of an approximate and preliminary nature, in 1928, were made with a single oscillator and a reflector to create the stationary waves (2).

It is well known that if the acoustic resistances differ greatly for two media the reflection coefficient for sound travelling in the one medium and incident on the other is high. Consequently, as water was the first liquid used, it was decided to employ an air reflector to create the stationary waves, for the acoustic resistances of air and water differ very greatly. The air reflector, Fig. 2, consisted of a flat, thin, sheet of mica *M*, fitted over the mouth of a bell-shaped piece of metal at the end of an open tube *TT*. The bell was of such a size that it fitted snugly into the experimental tube. The electric power applied to the oscillating circuit was rectified 60-cycle a.c. which generated in the oscillating circuit a "tonic train" of ultrasonic waves. Though the high frequencies employed were usually ultra-audible in pitch, the 120-cycle note of this tonic train could easily be distinguished by means of a stethoscope attached to the end of the listening tube *TT* projecting from the reflector.

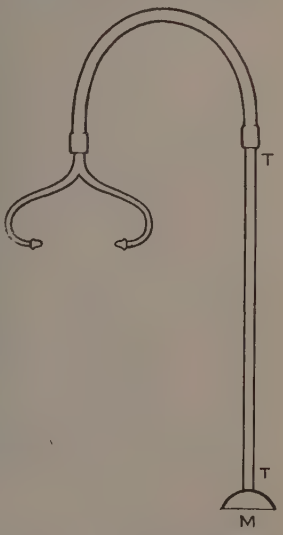

FIG. 2. *Air reflector, shown attached to stethoscope.*

Measurements of the wave-lengths were made by adjusting the position of the reflector in the experimental tube until the sound of the tonic train was a maximum. In this condition the column of liquid between the oscillator and reflector was in resonant vibration, a node of velocity occurring at the mica sheet. The position of the reflector with respect to a measuring scale was noted and then adjusted to the next maximum, which was, of course, one-half wave-length distant from the first. This process was carried out throughout the whole length of the tube, or in the case of extra long tubes, until the distance from the source of the waves became so great that the points of detectable maximum amplitude became indistinct. The velocity of the ultrasound in the liquid was determined by measuring the frequency of the generating electrical oscillations with a Hertzian wave-meter and calculating the velocity from the simple wave relation $v = n\lambda$.

It was at first intended to employ tubes of small rigidity; consequently the first trials were carried out with tubes of sheet celluloid, 0.04 cm. thick, made by rolling the sheets into a cylindrical form of single thickness and cementing together the overlapping ends. A few tubes of mica, made from mica sheets, were also used, but this material broke too easily when rolled into tubes of small diameter. In the first trials the experimental tube was set up vertically, the ultrasonic oscillator being held at the bottom end.

At very high frequencies the nodes and antinodes of the stationary waves were found to be very distinct, and the measured phase velocity was about the same as the unconfined velocity in a large body of the liquid. A small reduc-

tion in velocity might have been possible, but it was soon realized that with a wave-length short in comparison with the diameter of the tube, the velocity in the column of liquid was the same as in an unconfined volume. As the wave-length increased however, *i.e.*, as the frequency diminished, the phase velocity as measured by the stationary waves *was found to increase markedly, and the ease with which the nodes and antinodes could be distinguished became decidedly less.* The readings taken in a typical case are given in Table I, and a curve of velocity against frequency is plotted in Fig. 3. At frequencies lower than those quoted, standing waves could not be detected in tubes of this diameter, *viz.*, 2 cm. Several curves similar to this one were obtained.

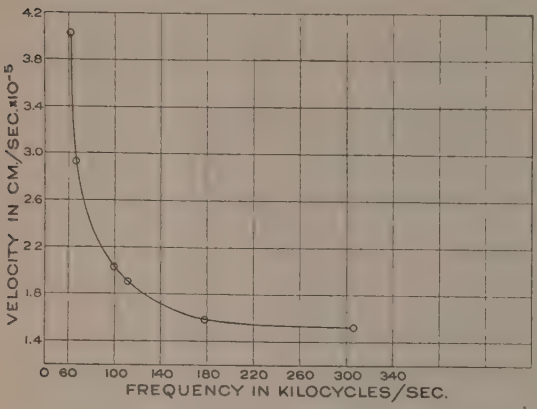

FIG. 3. *Curve of phase velocity against frequency. Water in tube made of celluloid sheet; internal diameter of tube, 2.0 cm.; wall thickness, about 0.4 mm.; temp., 18°C.*

TABLE I

EXPERIMENTAL PHASE VELOCITIES FOR A NUMBER OF FREQUENCIES

No. of antinodes observed	Frequency in cycles/sec.	Wave-length in cm.	Velocity in cm./sec.	No. of antinodes observed	Frequency in cycles/sec.	Wave-length in cm.	Velocity in cm./sec.
31	306,000	0.496	1.52×10^5	11	99,500	2.03	2.02×10^5
21	178,000	0.888	1.58×10^5	9	66,000	4.44	2.93×10^5
10	111,300	1.71	1.90×10^5	4	61,500	6.65	4.02×10^5

NOTE:— *Tube: Celluloid sheet; internal diameter, 2.0 cm. Wall thickness, about 0.04 cm. Liquid used—water. Temperature, 18° C.*

Results similar to the above were obtained on changing slightly the details of the experiment, though not its principle. For example, rubber and other materials were employed as walls of the experimental tubes, or the tubes were made more accurately cylindrical, or other "listening" detection devices were employed, such as a hollow steel reflector having a small central hole covered with a thin mica disk. Slight irregularities of wall thickness or of sectional shape made no great difference. The velocity-frequency curve was always of the same type as that of Fig. 3, within the range of detectable stationary waves. But it was observed that as the internal diameters of the experimental tubes were increased, though the velocity-frequency curves retained the form of Fig. 3, the high values of velocity were shifted towards the lower frequencies.

Further experiment soon showed it possible to detect stationary waves at frequencies lower than those corresponding to the higher velocities, for any

particular diameter of liquid column. For example, in the case of a celluloid ("pyralin") tube with water as the liquid, the velocity-frequency curve was as shown in Fig. 4.

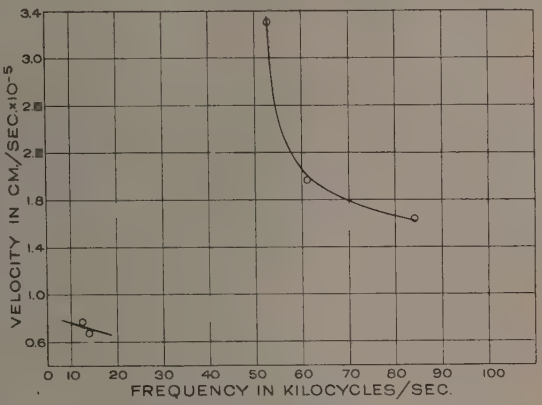

FIG. 4. *Curve of phase velocities for a number of frequencies showing both low and high values of velocity. Water in a pyralin tube, of internal diameter, 2.0 cm.; wall thickness, 1.5 mm.; temp., 18°C.*

The important points to notice about the curve in Fig. 4 are: (1) near the high velocity values *on the lower frequency side* there is a gap in the curve which could not be filled in by any observations depending on the detection of stationary waves; (2) at the frequencies below this region where stationary waves again existed, the velocities were markedly low; (3) the curve is similar in type to the selective absorption ("anomalous dispersion") curve of optics. Other curves similar to Fig. 4 were obtained, but in spite of many experiments it was not found possible by observations to map the curve more completely. Consequently other and better methods of experiment were devised.

Method I

An experimental tube of glass of internal diameter 3.5 cm., lengths varying from 10 to 30 cm., was set up horizontally, and a rod oscillator fitted into one end. The other extremity of the tube projected through a hole in one end of a small tank of dimensions 60 by 8 by 6 cm. (width), and a reflector of metal was placed in the tank at some distance from the mouth of the intruding tube. The tank and the tube were filled with water charged with cinder dust. A diagram of the arrangement is shown in Fig. 5. When the ultrasonic oscillator was operated the cinders formed stationary dust figures (3) in the glass tube, and on sifting more dust into the water in the tank, figures of stationary waves were easily disclosed in front of the reflector. Measurements of the velocity in the tube and in the tank were taken in this way, and compared with those taken by the listener method. The resulting velocity-frequency curves for the tube were the same from both dust figure and listener methods, and similar to the one shown in Fig. 4. However it is important to note that whereas, in the tank, the stationary wave dust figures were formed and the velocity was found to be the same at all

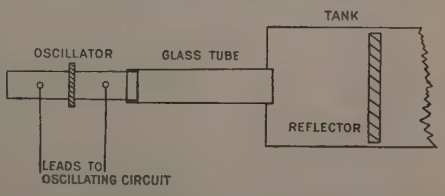

FIG. 5. *Dust figure method for showing confined and unconfined phase velocities.*

frequencies, in the tube the dust figures would not form at those frequencies at which stationary waves could not be detected in the tube by the former "listening" method. At these frequencies stationary waves in the tube apparently did not exist.

Incidental

A special narrow tank was constructed with a rectangular cross section and with ends and bottom of wood and sides of celluloid. The dimensions were: length of tank, 70 cm.; height, 8 cm.; and width, 3 cm. The end of a rod oscillator, of 1.9 cm. diameter, intruded through a hole in one end of the tank, and a movable metal reflector to form stationary waves could be placed in the tank at any desired position. The liquid in the tank was water. By means of both dust figure and listening methods the stationary wave field was surveyed. The resulting velocity-frequency curve was the same by both methods and was very much like that of Fig. 4. In this case the cross section of the liquid column was not circular, nor was the liquid completely surrounded with a wall, which showed that the great variation of velocity with frequency was independent of the sectional form of the liquid column, and of its partial or complete enclosure by a wall.

Method II

In experimental methods based on stationary waves it is often advantageous *to see* the representations of nodes and antinodes from which the measurements are taken. Consequently in the further course of this research it was decided to use tubes with *transparent* walls and to take advantage of the phenomenon of *ultrasonic cavitation* to make clear the position of the nodes and antinodes in the tube. In this method bubbles of gas produced in the liquid by the ultrasonic energy form curtains marking the nodes of the stationaries, and one can easily see when the wave-lengths are shortened or lengthened. There is here also an advantage over the dust figure method in that the present method avoids the necessity of adding dust or other impurities to the liquid.

If, at any given pressure in a liquid containing dissolved gas, stationary waves exist and are sufficiently energetic, small bubbles of gas form throughout the liquid and are driven to the nodes of displacement by the pressure of radiation of the waves. If an ultrasonic beam be directed vertically upward through the liquid column, layers of these bubbles will be formed, one-half wave-length apart, parallel to a horizontal reflecting surface; if the beam and column are horizontal the bubbles will be driven to the nodes and rise in vertical curtains, one-half wave-length apart, in the nodal planes (1, 4).

Naphtha is a liquid easily made to bubble (4) by cavitation, consequently it was now employed as an experimental liquid, enclosed in either glass or pyralin tubes. The tubes were set up vertically at first, the length of the naphtha column being adjustable. Stationaries were produced by reflection at the free air-liquid surface, and the wave-lengths measured by the distance between parallel layers of bubbles (1, 4). The results of a typical experiment are plotted in Fig. 6. It will be noticed that the gap shown in previous velocity-frequency curves is now more completely filled, but it was observed

that only *a very few regular nodes* could be detected at frequencies near those of the minimum or maximum velocities of the curve. Any formation of stationaries in this frequency range was very poor; the nodes were few and also irregular.

The curve of Fig. 6 bears a resemblance to the well-known selective absorption ("anomalous dispersion") curve of optics where the index of refraction (which is proportional to the reciprocal of the velocity) is plotted on a wave-length base. Although in Fig. 6 velocity has been plotted against frequency, this results in the same form of curve as is obtained by plotting reciprocal of velocity against reciprocal of frequency (which is proportional to wave-length). Hence the two curves are comparable, and the characteristic fall, sharp rise and further fall are unmistakable. Selective dispersion is caused by the selective absorption of energy at the frequency of the sharp discontinuity of the velocity-frequency (or reciprocal of velocity and frequency) curve. It thus appears that in the present case there is a special absorption or transference of energy at and near the frequency of the maximum in the velocity-frequency curve of Fig. 6. Under such conditions of absorption it would be difficult or impossible for stationary waves to form in the tube.

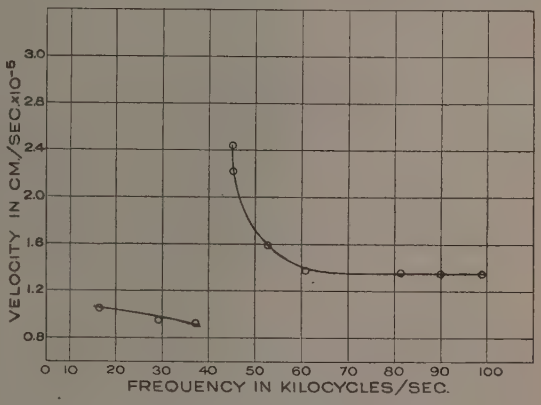

FIG. 6. *Complete curve of phase velocities for a number of frequencies; naphtha in a glass tube.*

Thus the problem of this research developed into an investigation to determine how the energy was absorbed at the "absorption frequency" and how this frequency depended on the dimensions of the column of liquid, the dimensions of the tube walls, and on the materials of both. For this purpose an improved method of experiment was devised.

Method III

By using two transmitters to produce the stationary waves, one at each end of the experimental tube, instead of relying on the reflection by a single reflector at one end only, it was found that more perfect stationaries could be produced, especially at the troublesome frequencies of stationary wave measurement near the minimum and maximum of the velocity-frequency curve. Identical transmitters, connected *in parallel* to the oscillating electrical circuit, were fitted into the opposite ends of the experimental tube (2). This tube was provided with a delivery tube of small diameter near the middle, through which the experimental tube could be filled with the experimental liquid, and to which a vacuum pump could be attached to facilitate cavitation in the liquid

by lessening the internal pressure. The arrangement is shown in Fig. 7. The distance between the transmitters could be varied slightly, but this had little effect on the stationary waves unless the tube was so long that there could be a significant loss of ultrasonic energy intensity between the tube ends and its centre.

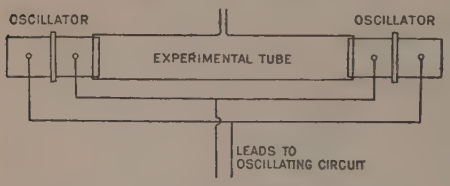

FIG. 7. *Final experimental arrangement.*

That this method of experiment was a great improvement on the former methods could be seen in the fact that more and better-formed stationary waves could be detected at the difficult frequencies in the vicinity of the maximum and minimum of the velocity-frequency curve.

Various Liquids

The position of the break in the velocity-frequency curve, in other words the absorption frequency, for any given diameter of the experimental tube would be expected to depend on the contained liquid, and, for any selected liquid, to depend on the diameter of the tube.

Experiments illustrating these points were carried out on water, naphtha, castor oil, transformer oil, and chloroform, under the same experimental conditions and consecutively in the same experimental tube. As examples, results for naphtha and transformer oil are shown graphically in Fig. 8. Changes in the energy intensity of the ultrasound emitted from the oscillators made no appreciable difference in the results: on occasions the high frequency voltage applied to the oscillators was doubled, thereby increasing the ultrasonic energy intensity about fourfold, but without any noticeable effects on the velocity-frequency curves. Transformer oil was found to be a very convenient liquid in experiments such as these, for the bubbles produced in it by cavitation were finer and rose through the liquid more slowly, owing to its greater viscosity.

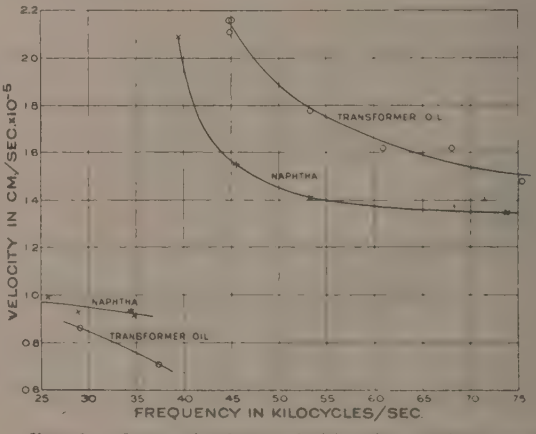

FIG. 8. *Curve of phase velocities for naphtha and transformer oil; transformer oil in glass tube of internal diameter 3.1 cm. and wall thickness, 1 mm. Naphtha in glass tube of internal diameter 3.5 cm., and wall thickness, 2 mm.*

Influence of Diameter of Column

An interesting experiment showed the variation in length of stationary waves along a tube in which the diameter varied.

A conical glass tube of dimensions: internal diameter at large end, 6.60 cm., at small end, 4.80 cm.; length of tube, 39.8 cm.; thickness of glass wall, 2.0 mm., was set up vertically, and a rod oscillator was fitted, as formerly described, into its smaller end. This tube was filled with transformer oil and stationary waves were produced by reflection from a horizontal plate in the oil. When the oscillator was operated at fairly high frequencies no difference could be detected in the lengths of the standing waves, but at a certain lower frequency notable differences in lengths of stationary waves occurred. In Table II the distance between successive nodes with corresponding computed phase velocity is given as well as the mean internal diameter of the tube corresponding to this particular half-wave-length. The results were anticipated from the previous work.

TABLE II

EXPERIMENTAL RESULTS AT A FREQUENCY OF VIBRATION OF 26,170 CYCLES PER SEC.

Distance between nodes half-wave-length in cm.	Corresponding velocity in cm./sec.	Mean diameter over the half-wave in cm.	Distance between nodes half-wave-length in cm.	Corresponding velocity in cm./sec.	Mean diameter over the half-wave in cm.
1.90	0.995×10^5	4.87	3.88	2.03×10^5	5.59
2.00	1.05×10^5	4.36	3.75	1.96×10^5	5.77
2.30	1.20×10^5	5.01	3.58	1.87×10^5	5.93
3.70	1.94×10^5	5.19	3.48	1.82×10^5	6.09
3.88	2.00×10^5	5.37	3.10	1.66×10^5	6.24

In another experiment a glass tube was drawn into the shape shown in Fig. 9.

The internal diameter of the larger sections was 3.0 cm.; that of the smaller 1.7 cm. It was found difficult at most frequencies for stationary waves to form in the smaller section of the tube, but there were three different frequencies at which stationary wave measurements were quite possible. At a frequency of 51,800 cycles per sec. the wave-length in the large sections was 4.40 cm. and in the small section approximately 3 cm.; at 81,700 cycles per sec. the wave-length in the large sections was 1.91 cm. and in the small 2.1 cm.; at 93,600 the wave-length in the large section was 1.67 cm., and in the small 1.86 cm. It is seen clearly from this simple experiment that in a given liquid the phase velocity depends upon (1) the frequency and (2) the diameter of the column, and that the frequency may be so adjusted that the phase velocity is greater in the larger tube than in the smaller, or *vice versa*.

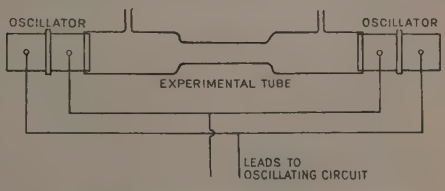

FIG. 9. *Experimental tube of different diameters.*

A series of experiments using transformer oil as the experimental liquid was carried out with glass tubes of the same length and wall thickness, but with different diameters. In Fig. 10 are plotted the frequencies at which the maxi-

mum observable phase velocities occurred in the chosen diameters of tube. On the same sheet has been drawn the curve $n_p = \frac{K}{d}$, where n_p is the absorbing frequency in thousands of cycles per sec., d is the diameter of the tube in cm., and K is a constant (in this case having the value 13.6). It will be observed that a smooth curve drawn through the experimental points would lie approximately on the curve which has been drawn. It is very difficult to obtain n_p experimentally, solely from velocity measurements, since the velocity is asymptotic to the ordinate at the frequency n_p, and the greatest observable velocity may be at a frequency somewhat removed from n_p. But from Fig. 10 there is a striking indication that *the absorbing frequency depends inversely on the diameter of the tube.*

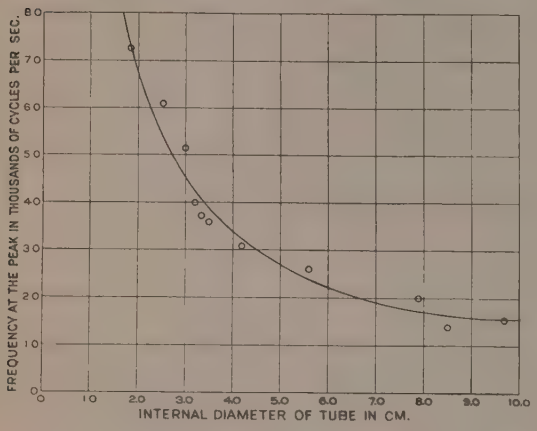

FIG. 10. *Curve showing relation between absorption frequency and internal diameter of tube; transformer oil in glass tubes. The smooth curve represents the relation, peak frequency* $= \frac{K}{\text{diameter of tube}}$, *where K was taken as 13.6. The observed frequencies were plotted as circles.*

Influence of Length of Tube

To determine whether or not longitudinal resonance in the tube wall, as suggested by Dörsing, had any significant influence on this phenomenon or was a possible cause of the marked energy absorption, special experiments were begun with a piece of glass tubing, about 56 cm. long. This tube was shortened many times and the velocity-frequency curve for the contained liquid was retaken at each length. The shortening was by amounts of about $\frac{1}{8}$ wave-length of longitudinal wave in the wall, at the peak frequency of the velocity curve, for a range over a full wave-length, and after that at irregular intervals, down to a length of 30 cm. It was noted that the velocity-frequency curve remained exactly the same for all the lengths of experimental tube. Herein is a variance from the observations of Dörsing (7), though it must be remembered that he experimented with liquid columns at the much lower frequencies of about 4000 cycles per sec. Dörsing suggested that the vibrating liquid columns generate sympathetic longitudinal vibrations in the tube wall, as undoubtedly they may do, and that to obtain Kundt's dust figures one must make the natural frequencies of liquid and tube as nearly equal as possible. The length of the experimental tube in relation to the length of the wave within the wall or within the contained liquid made no difference here to the formation of the stationary waves, as indicated by the bubbles produced by cavitation in the nodal planes.

A special experiment was later performed with another glass tube, internal diameter 3.1 cm., using oscillators which fitted very snugly into the ends of the experimental tube and which were driven with maximum power at their fundamental resonant frequency; and it was arranged that this frequency was the same as the frequency of the maximum of the velocity-frequency curve. Under such conditions vibrations must have been very readily communicated to the tube walls, but there were no noticeable differences in the experimental results. (The experimental liquid in this particular case was transformer oil.) If there were any shift of the velocity peak for different lengths of tube it was very small indeed, and could not account for the *large difference in peak velocities noticed for tubes of different diameters.*

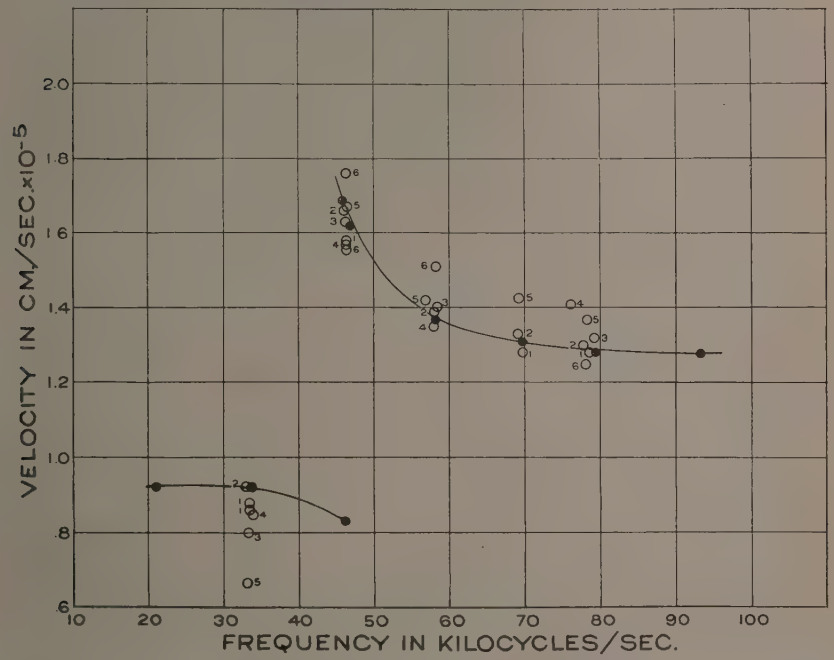

FIG. 11. *Curve showing effect of varying length of tube on the phase velocity. Naphtha in glass tubes, 3.1 cm. internal diameter; wall thickness, 1.4 mm. Points represent length of tube as follows:—*

25 cm. (●), 18.5 cm. (○₁), 13.4 cm. (○₂), 9.1 cm. (○₃), 6.6 cm. (○₄), 4.5 cm. (○₅), 3.2 cm. (○₆).

Very short tubes are commonly employed in sonic interferometer measurements, and it was desired to ascertain definitely whether the velocity-frequency curve would remain unchanged down to extremely short lengths of tube. Hence it was decided to measure velocities in tubes of even shorter length than those formerly used. The liquid employed was lighting naphtha contained in glass tubes of internal diameter 3.2 cm. and greatest length 25 cm. The experimental results are presented in graphical form in Fig. 11.

It will be noticed that down to a tube length of 9.1 cm. (points marked "3" on curve) the observed velocities are the same as those obtained with a tube length of 25 cm., but below 9.1 cm. tube-length, the points depart from the 25-cm. curve in an erratic manner. The rise at 46,000 cycles per sec. however, is quite well defined in all cases, so that the frequency of the absorption band seems to persist unchanged right down to a tube length of 3.2 cm. The deviations from the 25-cm. curve at the higher frequencies are in all probability due to the small number of wave-lengths between the opposing oscillator faces, and the consequent difficulty in accurately measuring wave-lengths.

Effect of Different Thicknesses and Materials of Tube Walls

To determine what effect, if any, the thickness and material of the tube wall had upon the velocity in the liquid, a number of experiments were conducted. Four glass tubes of the same internal diameter, 3.1 cm., and of ordinary thin but different wall thicknesses were obtained and readings of velocity were taken in transformer oil at frequencies near that of the maximum. In all cases the velocity- frequency curve was the same.

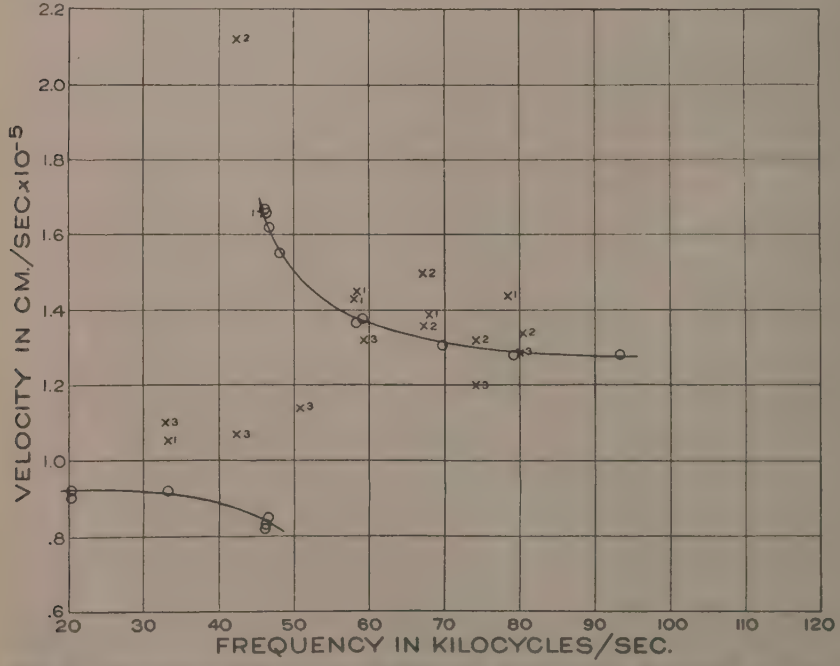

FIG. 12. *Curve showing influence of wall thickness on phase velocity. Naphtha in glass tube; internal diameter of tube, 3.1-3.2 cm. Wall thickness, 1.4 mm.;* $\frac{h}{a} = 0.087$, *points plotted as* (○). *Wall thickness, 2.7-3.0 mm.;* $\frac{h}{a} = 0.18$; *points plotted as* (X1) *and* (X2), *the numerals indicating that the readings were taken on different days. Wall thickness, 0.33 mm.;* $\frac{h}{a} = 0.21$, *points plotted as* (X3). *In this case the nodes were scarcely defined at all, and the readings are very doubtful.*

A thin-walled celluloid tube and a very thin cellophane tube were constructed and filled with lighting naphtha. Readings of velocity were taken and compared with those taken in a glass tube of the same internal diameter but different wall thickness. In these cases also there was no change.

Further readings were taken when using lighting naphtha in tubes of glass, two with quite thin walls and two with comparatively thick walls. Here different results were obtained. The calculated velocities are plotted in Figs. 12 and 13.

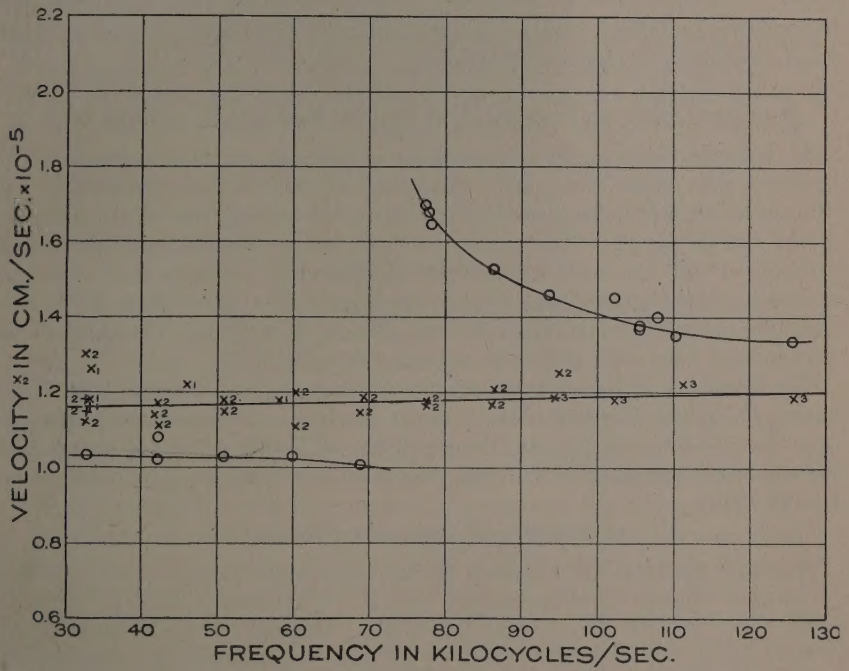

FIG. 13. *Curve showing influence of wall thickness on phase velocity. Naphtha in glass tubes of internal diameter 1.9 cm.; wall thickness, 1.2 mm.;* $\frac{h}{a} = 0.13$*; points plotted as circles. Wall thickness, 3.3 mm.,* $\frac{h}{a} = 0.33$*; points plotted as crosses. The readings were taken on different days as indicated by the numerals alongside the crosses.*

Evidently in wide tubes of relatively thin walls the thickness of the wall and its material has no influence on the absorption frequency and the velocity; but with a given tube material, *when the wall thickness becomes appreciable in comparison with the radius of the liquid column*, the whole phenomenon is greatly affected. The standing wave system is very unstable and velocities are difficult to measure, and seem to be about midway between the low velocity value at lower frequencies and the asymptotic value of velocity at higher frequencies.

These results are very interesting. In Fig. 12, the velocity curve is quite

regular for a ratio of wall thickness (h) to radius (a) of 0.087 $\left(=\frac{0.14}{1.6}\right)$. When this ratio equals 0.18 $\left(=\frac{0.29}{1.6}\right)$ the velocity becomes erratic though the absorption peak is unaltered. For a value of the ratio of 0.21 $\left(=\frac{0.33}{1.6}\right)$, the peak has almost disappeared and the velocity is very difficult to measure.

In Fig. 13 the velocity curve is regular for $\frac{h}{a}$ equal to 0.13 $\left(=\frac{0.12}{0.95}\right)$. When this ratio equals 0.35 $\left(=\frac{0.33}{0.95}\right)$ the velocity shows no trace of a peak and the velocity, although erratic, is beginning to steady down. Further work will be necessary to elucidate this point.

Further Check on Possibility of Lateral Resonance in Tube Wall

As a further experiment to determine whether or not lateral resonance in the tube wall might be affecting the velocity in the contained liquid, it was decided to use tourmaline instead of quartz, as the active piezo-electric material of the rod oscillators. The reason for using tourmaline is that this crystal vibrates in only one main direction of oscillation for a voltage applied in that direction, while quartz may vibrate in three. The tourmaline oscillators, therefore, reduced to a minimum the chance of a lateral vibration being transmitted from the oscillator rods to the tube walls.

The velocities obtained with quartz and tourmaline oscillators were compared, and it was observed that the same velocities in the experimental liquids occurred in both cases, so that the possibility of lateral resonance in the tube wall having anything to do with the absorption phenomenon under observation is very slight.

Absorption at Overtone Frequencies

Attempts were made to find other peaks ("absorption bands") in the velocity-frequency curve. It was thought that if other peaks existed in the curve such might occur at overtones of the frequency of the first, since the effect discussed here was no doubt due to selective resonance of oscillation in some possible degree of freedom. Accordingly in a special experiment, in a glass tube containing naphtha, readings of velocity and frequency were taken up to quite a high frequency. The resulting curve is shown in Fig. 14. It will be seen that the first absorp-

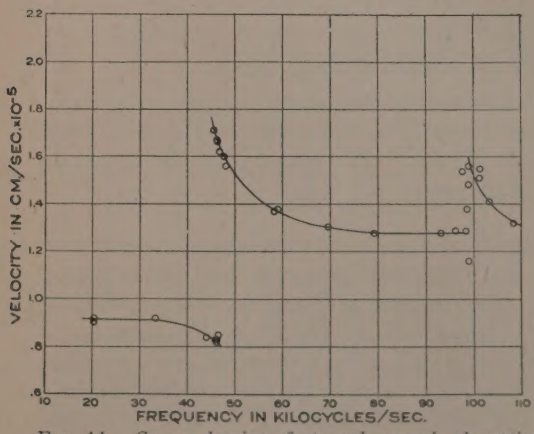

FIG. 14. *Curve showing first and second absorption bands: naphtha in glass tube, 3.1 cm., internal diameter; wall thickness, 1.4 mm.*

tion band existed at a frequency of about 45,000 cycles per sec., and that there is a second band at a frequency of about 100,000 cycles. The depressed velocity observed before the first absorption band seemed to be missing, but the sharp fall in velocity after the absorption frequency is as usual. The experiment shows clearly that more than one absorption frequency, probably existing in a frequency series, may occur in a liquid in the same tube.

Incidental

Velocity in Air

The velocity of sound in air was measured in several tubes by using a hollow steel reflector with attached stethoscope as in the earlier experiments with liquids. No differences in the velocity in the air columns could be found at different frequencies, and the velocities so measured agreed with the unconfined velocity in air at the same temperature. This, however, does not exclude the possibility of selective absorption bands occurring in a gas column when other relations between the nature of the gas, diameter of column, and wave-length may prevail.

Conclusion of Part I

It is evident from these experiments that at certain particular frequencies, for which there exists some relation between the diameter of the tube and the wave-length of the oscillation in the fluid column enclosed by the tube, there is a marked selective absorption of energy which displays all the usual characteristics of an energy absorption band. Phase-velocity is greatly lowered immediately on the lower side and greatly enhanced immediately on the higher side of the critical frequency. Similar would be the conditions of amplitude, particle velocity and harmonic pressure in the waves. No doubt it is at frequencies far removed from the absorption where the wave-length is either very large or very small compared with the diameter of the column that all other experiments on velocity have been carried out; *i.e.*, on the regular and flat portions of the velocity-frequency curves, far removed from the frequency of the discontinuity. It is, no doubt, in these ranges that theories like the Helmholtz-Kirckhoff theory may be applied. At or near the frequency of absorption the velocity changes represented by such theories are relatively insignificant.

The fact that the absorption frequency does not depend on the material or length of the tube, or (for thin walls) on the wall thickness, indicates that it is neither longitudinal nor flexural (lateral) vibrations in the tube walls which cause the phenomenon; and the fact that for any liquid the critical frequency shifts with change of diameter indicates that it is in the column of liquid itself that the energy absorption or transference takes place.

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